Evolution of tolerance:
its influence on the ability of remote sensing to detect contaminated ground via vegetation stress

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Declaration

I declare that this thesis, which I submit for the degree of Doctor of Philosophy at the University of Edinburgh, was composed by myself and is my own work.

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Abstract

The detection of contaminated ground, such as metal contamination is desirable to locate contamination events, determine land use and for mineral exploration using geobotany. Remote sensing users currently attempt to use spectral indicators in plants to locate contaminated ground via stress effects on vegetation. However, up to now this approach has ignored the phenomenon of evolution of metal tolerance in some plant species. Some species are metal tolerant, as well as some populations within a species. Tolerant plants may dominate the community on contaminated ground, and may not exhibit the conventional stress spectral signatures, thus limiting the applicability of the approach. This study investigated the problem through an analysis of reflectance data from experimental plots.

Metal tolerant and non-tolerant plants from two grass species (*Festuca rubra* and *Agrostis capillaris*) were grown in standardised conditions with control, copper, zinc and salt (NaCl) treatments. Leaf reflectance properties (400 - 1100 nm) of the different treatments were measured using an integrating sphere. Pigment concentration analysis was also undertaken. Differences between treatments were examined using the raw reflectances, as well as a number of published and novel vegetation indices and red edge position. Non-parametric statistical tests were used. Tolerant plants showed different spectral responses to non-tolerant plants irrespective of treatment. No spectral analysis technique consistently showed a stress response in all non-tolerant treatments, although different techniques did show different stress responses.

In the second part of the study canopies of monocultures and mixtures of tolerant and non-tolerant ecotypes of *Festuca rubra* were grown in plots with control and zinc treatments. Reflectance measurements (400 - 2500 nm) were made under artificial lighting using a spectroradiometer mounted inside a light proof tent. Pigment analysis was also undertaken. Spectral analysis techniques were the same as those used in the first part of the study. No vegetation index gathered from the literature distinguished the control from the metal treatments for the monoculture plots, but many were successful for the mixture plots. Indices developed during this study based on the green-red region were successful. Red edge position bore no useful relationship to treatment. Non-tolerant stressed plants showed an early senescence, which may be crucial to their detection.

The addition of metal contamination to the natural environment will typically stress non-tolerant plants that are present, and tolerant plants may become dominant in the community. Leaf and canopy reflectance models were used to simulate reflectance changes following community responses to metal stress. These showed that the detection of contaminated ground via vegetation stress requires that the area being surveyed is either comprised of a non-tolerant ecotype which is stressed (i.e. before being outcompeted by tolerant plants) or that the contamination level is so high the tolerant ecotype shows stress.

The results indicate that the use of remote sensing to detect contaminated ground via vegetation stress is far from straightforward. To be successful the measurements have to be made during a community transition from non-tolerant stressed plants to tolerant plants (although this transition could vary from weeks to years). The measurements should also be made over the senescence time period, to locate areas senescing early. As many methods of analysis (indices, red edge position etc.) as possible should be used. The users also have to accept that false negative results will occur where tolerant populations are on contaminated ground. The cause of stress will not be identifiable.

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Chapter 1: Introduction

1.1 Context

Remote sensing could offer many benefits to the location of contaminated ground. Its ability to monitor wide areas at high spatial resolution would enable users to track areas around industrial sites, as well as explore for mineral ore. It could be used to directly monitor soil or rock composition where it is exposed, and to monitor plant stress where it covers metal sites as a symptom of below surface contamination (Goetz *et al.*, 1983), (Cannon, 1971). However, the use of plants as biomarkers of contamination has aspects which have largely been ignored by remote sensing users. Two such aspects include the lack of a direct relationship between soil metal content and plant stress and the fact that some plant species have evolved metal tolerance. This study investigates the relationships between soil metal content and the reflectance of plants of different tolerance.

Biomarkers, as defined here, are organisms that respond to a stressor in such a way that they provide a measure of the availability of the stressor in the environment. The use of biomarkers relies upon the identity of the species being known, knowledge of the physiological responses of that species to stress, and the equal response of all members of that species. The most successful use of biomarkers has been in laboratories where controlled populations may be kept under controlled conditions and exposed to controlled doses of stressors. In the natural environment their use is more limited as individuals of the same species vary in their responses. The best uses of biomarkers in the wild have involved community level investigations where the presence and response of a range of species are used to signify a contaminated environment (e.g. river surveys or geobotany (Walker *et al.*, 1997), (Canon, 1960)). Remote sensing cannot identify species (Price, 1994), and its use of biomarkers relies upon the physiological response of all species to stress being the same.

The assumptions that remote sensing makes for all plants are shown in Figure 1.01. These relationships have been simplified, and in reality are not necessarily positive or linear. Instead they should be taken as indicating values further away from those at low "x" axis values. Remote sensing assumes that:

- There is a relationship between metal concentration and plant stress (Fig. 1.1a). With higher metal concentrations plants are more stressed.
- There is a relationship between plant stress and a spectral signature (Fig. 1.1b). A spectral signature could be simple reflectance, or an index or measure of the shape of the reflectance spectrum.
- Given the two above assumptions, it is thus assumed that there is a relationship between metal concentration and a spectral signature (Fig. 1.1c).

Metal tolerance in plants is a widely studied phenomenon (e.g. Antonovics *et al.*, 1971, Baker, 1987). It has evolved in some species, and within a species visually identical populations (ecotypes) can vary in tolerance. There is natural variation for metal tolerance in some species which is selected for on contaminated sites. These individuals will have different responses to metals than non-tolerant individuals. They may only be stressed by higher levels of contamination than non-tolerant plants, or they may even have higher requirements for essential metals and so be stressed by low metal concentrations (hereafter called "deficient" plants). The responses of tolerant and deficient plants are different to non-tolerant plants (Fig. 1.1 "Actual responses" series). They:

- may only be stressed by higher metal concentrations ("Tolerant") or be stressed by low and high levels of metal, and unstressed at medium levels ("Deficient"; Fig. 1.1.A).
- may show the same spectral response to stress although they will become stressed at different levels (Fig. 1.1B).
- may show different spectral responses to the same metal concentration (Fig. 1.1C). Thus the same spectral response (Fig. 1.1C line R) could indicate "deficient" plants at low metal levels, non-tolerant plants at medium metal levels, or tolerant and deficient plants at high metal levels.

The description of plants as tolerant or non-tolerant is inadequate to define the range of tolerance within and between species. Where they can, plants evolve tolerance to suit their environment, and tolerant plants preferentially inhabit contaminated areas (Grime, 2001). Tolerance to a recent stress event on a site can occur in less than 6 years (Nicholls and McNeilly, 1979). Therefore contaminated ground is likely to have tolerant populations which have a different stress response to non-tolerant plants. In remote sensing, the use of plants as biomarkers of metal contamination, and the reliance on all plants having the same stress and spectral response to a particular metal concentration, must be tested.

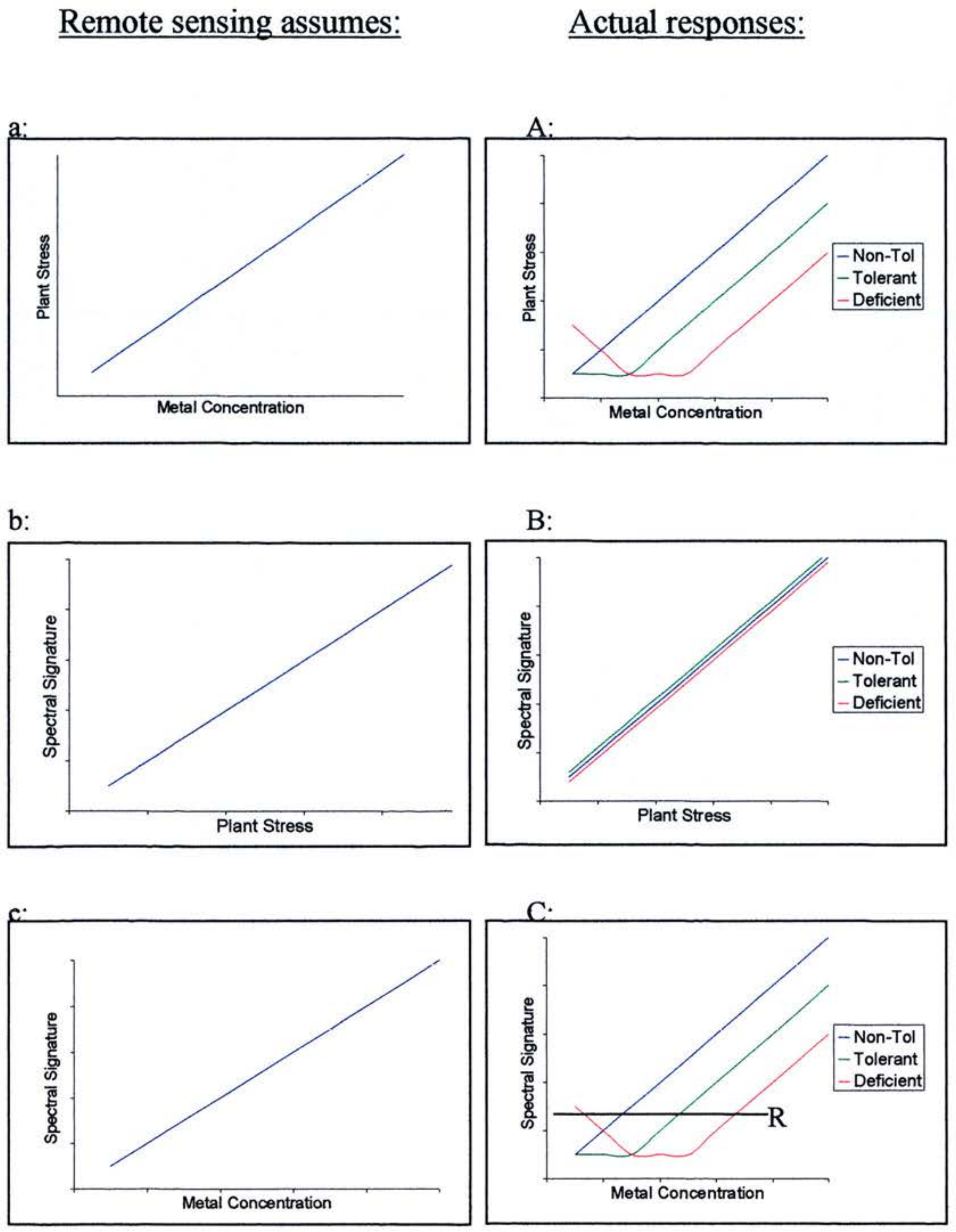

Figure 1.01. Remote sensing's use of vegetation as biomarkers assumes the above relationship between metal concentration and plant stress (a), the spectral signature and plant stress (b), and inferred from (a) and (b) that there is a relationship between the spectral signature and metal concentration (c). The right hand column shows the actual response between these factors in the natural environment where there are plants that are non-tolerant and tolerant of the stress, as well as plants that may require that stressor to grow normally. All relationships are simplified to be positive linear ones. For an explanation of R, refer to the text.

1.2 Aims

This study investigated the leaf and canopy reflectance of tolerant and non-tolerant grasses subjected to metal stress. The central aim of this study was to test the ability of remote sensing to detect metal stress in plants of different tolerances. Techniques used were red edge position, relevant vegetation indices cited in the literature and vegetation indices developed during this study.

Specific aims were:

- To test for a stress reflectance response in non-tolerant plants. As most techniques have been developed empirically, this was to test for transferability to the species used here.
- To investigate whether there was a stress response in tolerant plants. Very few remote sensing studies have considered tolerance.
- To develop novel remote sensing techniques for discriminating stress in plants.
- To model community composition changes that occur on recently stressed sites to investigate their simulated reflectance response.
- To propose a technique or number of techniques that can identify stress regardless of community composition. If this is not possible, to advise the best techniques and study regime to use.

The relationship between soil metal content and plant physiology is reviewed in Chapter 2. Chapter 3 describes how the physiology of plants affects their reflectance response. Techniques used in remote sensing to identify stressed plants are also considered. Chapter 4 examines the leaf reflectance response of tolerant and non-tolerant plants of two species to three different stressors (zinc, copper and salt). Remote sensing techniques already in use are investigated, as well as novel ones. Chapter 5 relates the results of canopy reflectance of tolerant and non-tolerant plants of one species to zinc contamination. Reflectance analysis techniques from the literature as well as novel ones were tested on this data. In Chapter 6 modelling techniques are reviewed, and a model of community change following a stress event is developed. This is used to model the reflectance of stressed communities using PROSPECT and SAIL models. Chapter 7 draws the main outcomes of this study together in the context of the validity of using remote sensing to locate contaminated ground.

1.3 References

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Chapter 2: Plant - Soil Interactions

2.1 Introduction

With remote sensing proposed as a method for detecting soil contamination via the biophysical responses of plants, the relationship between all of these factors needs to be investigated. This chapter reviews soil metal interactions, plant metal uptake, and plant community response. For remote sensing to be a valid technique, soil metal needs to relate to the metal available to a plant, available metal needs to relate to the uptake of metal by a plant, and the plant's level of metal uptake needs to relate to its physiological response. However, Soil - Plant relationships are complex and different populations of plants can tolerate metal concentrations that stress others. All of these factors are reviewed here, and as the remote sensing community is least familiar with the phenomenon of metal tolerance, it is that which is concentrated upon.

2.2 Metal Contaminated Soil

2.2.1 Introduction to soil

Soil is a mixture of mineral and organic compounds, and a below ground ecosystem which provides habitats for plants and animals and influences the characteristics and fluctuation of the community. Soil offers support, nutrients, water, oxygen, and a safe microclimate. It also harbours pathogens, root grazers, toxins, and may offer inadequate supplies of nutrients, water or oxygen. The soil climate provides a low temperature variation seasonally and diurnally, and is moist.

A "typical" soil can be taken as a number of layers, an organic rich horizon comprised of recently deposited litter, a mineral horizon comprised of decayed organic matter and minerals, and a mostly mineral horizon and bedrock (Figure 2.01). Organic matter is derived from decaying vegetative matter, while the mineral fraction derives from the erosion of local rock (Brady and Weil, 1996).

2.2.2 Metal Sources

Toxic heavy metal ions are ubiquitous in the environment, though normally at low levels (Meharg, 1994). High levels of metal contamination have been a localised factor around surface ore deposits for millennia, and has more recently been spread to previously unpolluted sites by anthropogenic activity. Disturbance of metal deposits, and their processing and use release metals into the environment. Traces of lead have been found in arctic ice cores dating back to Roman times, indicating the potential dispersal of metals (Baker, 1987). The extraction and use of metals increased slowly until the industrial revolution, then grew rapidly (Baker, 1987).

Today's quantity and range of metal usage is higher than ever. While present day extraction is at very high levels, the extraction process is also cleaner than in the past due to environmental regulation, at least in the developed world (Morrey *et al.*, 1984) . It is the processing and use that metals are put to which causes the most dispersal today. As more metal is in use the potential for contamination is higher across the whole landscape, rather than just around ore/mine sites, than at any time in the past. Contemporary inputs of metal into the environment and the respective metals involved are listed in Table 2.01. Although input levels may be low and may not cause toxic conditions at present, their continuing deposition combined with increasing soil acidity (with acid rain) may mean that the toxic threshold will be passed in the future. Metals do not degrade, so concentrations will build up over time even with low inputs. With increased applications of metals onto the landscape, identification of contaminated locations in the ephemeral landscape is very important. In Russia decades of unregulated industry have left a large burden of metals in soils, and with recent further development of industry, identification of contaminated sites is required (Kurkjian, 2000).

The source of contamination largely determines the metal's position in the soil (Table 2.02, (Baker, 1987; Ross, 1994a). Sites where the soil is derived from parent rock which contains metals will have the metal throughout its profile, whereas areas receiving inputs of metal (sludge, irrigation) will have the metal concentrated mostly in the surface (Ross, 1994a; Atkins *et al.*, 1982). Some sites have combinations of sources, e.g. Avonmouth, Bristol, where a motorway, incinerator and smelter all contribute to metal loading (Coughtrey and Martin, 1978). There are 50-100,000 industrial waste sites in the UK which are contaminated (Ross and Kaye, 1994). Furthermore, three quarters of all soil in the UK is contaminated to some extent (Macnair, 1993).

Table 2.01. Sources of heavy metals in the environment.

Source	Metal
Surface ore deposits	Any
Volcanoes	Any, especially Al, Mg, Fe
Spoil heaps – weathered	Any, especially As, Cd, Hg, Pb
Tailings dispersed fluvially, redeposited during flood, dredging etc.	Any, especially As, Cd, Hg, Pb
Transported ore – blown onto soil	Any, especially As, Cd, Hg, Pb
Smelting – aerosols and dust from stack	Any, especially As, Cd, Hg, Pb, Sb, Se
Iron and steel industries	Cu, Ni, Pb
Metal finishing	Zn, Cu, Ni, Cr, Cd
Plastics	Co, Cr, Cd, Hg
Textiles	Zn, Al, Z, Ti, Sn
Microelectronics	Cu, Ni, Cd, Zn, Sb
Wood preserving	Cu, Cr, As
Refineries	Pb, Ni, Cr
Urban/Industrial sources – incineration etc.	Cd, Cu, Pb, Sn, Hg, V
Pyrometallurgical industries	As, Cd, Cr, Cu, Mn, Ni, Pb, Sb, Tl, Zn,
Automobile exhausts	Mo, Pb, V
Fossil fuel combustion	As, Pb, Sb, Se, U, V, Zn, Cd, Ba, Cu, Mn
Fertilisers impurities	As, Cd, Mn, U, V, Zn, Cr, Mo, Pb,
Manures and Composts	As, Cu, Mn, Zn, Cd, Ni, Pb
Lime	As, Pb
Pesticides	Cu, Mn, Zn, As, Hg, Pb,
Irrigation waters	Cd, Pb, Se
Corrosion of metals – includes pylons	Fe, Pb, Zn
Sewage sludge	Many, especially Cd, Cr, Cu, Hg, Mn, Mo, Ni, Pb, V, Zn
Leachate from landfill	As, Cd, Fe. Pb
Scrapheaps	Cd, Cr, Cu, Pb, Zn
Bonfires	Cu, Pb

After Ross, 1994a; Al-Hiyaly *et al.*, 1990; Alloway and Ayres, 1993.

Table 2.02. How the source of metal affects its position and its effect on plants.

Variable	Mine / Ore soil	Recently Polluted
Age	>50 years	< 50 years
Concentration of metal	High	Low initially
Variation in concentration	Unchanging	Increasing
Spatial variation	Heterogeneous	Uniform
Distribution through soil	Uniform	More at the surface
Other factors	Deficient in nutrients and organic matter, a poor substrate for growth	"Normal" soil
Evolutionary force	Selection forces strong and stable	Selection forces weak and increasing

From (Baker, 1987).

Knowledge of where soil metal is present is necessary to locate ore sources, allow remediation and to determine land use so toxic metals do not affect human or ecosystem health. As metal sources are varied and can be wide ranging tracking inputs is not feasible (Table 2.01). Traditional methods of measuring soil pollution involve taking soil samples and measuring its metal content. This is feasible where pollution is known to have occurred, but not for the detection of recently contaminated areas. Metal content in soil can vary over very small areas, so many soil samples are needed to determine metal content for an area (Nicholls and McNeilly, 1979). Remote sensing potentially offers a rapid large area method of environmental assessment and for locating pollution sources saving considerable time and money on taking soil samples from sites.

2.2.3 Soil - Metal Interactions

The transfer of metals in soil to plants has a large number of variables, many of which have not been quantified in isolation:

Adsorption and exchange in soil – affected by colloids, pH and organic matter.

Mass flow and diffusion of metal ion – affected by water availability and plant uptake.

Precipitation of metal as solid – influenced by soil chemistry.

Root biomass and its distribution relative to the metal.

Microbial activity – affects nutrient cycling.

(Ross, 1994b).

Not all metal in soil is available to plants (i.e. bioavailable). The metal may be present in ionic or particulate form, although only the ionic form becomes bioavailable. Bioavailability is dependent on a number of factors. The source determines bioavailability to some extent as it determines the proximity of the metal to the plant roots. The metal's chemical characteristics and soil conditions determine it's chemical availability. The soil's characteristics may also modify a metal's chemical availability. Constituents of the soil may bind to the metal so rendering it unavailable (e.g. organic matter binds Cu strongly), or chemical features may cause its release or precipitation. Different metals chemical properties mean they have different bioavailabilities, which is also determined by soil characteristics. Soil characteristics are not constant; nutrients are released in pulses, and seasonal effects, wetting and drying, freezing and thawing, may vary in scale with different mechanisms. Increasing soil pH can increase or decrease different metals availabilities depending on the characteristics of the metal. Surface organic matter can mobilise or demobilise metals in the soil (Ross, 1994b). Organic matter can also improve the general characteristics of a mine soil by providing nutrients and increasing water retention (LeFebvre and Simon, 1979).

High metal concentrations also affect soil microorganisms. However, the effects on soil organism abundance is not simple, copper causes a reduction in soil bacteria, while cadmium and lead can affect bacteria, fungi, nematodes and earthworms (Ross and

Kaye, 1994). This in turn will reduce nutrient cycling, lowering the fertility of a site. Mine sites themselves differ from normal sites in more than just the amount of metals. Organic and nitrogen decomposition rates decrease with high metal concentrations (Ross and Kaye, 1994). Total N can be similar in mine and non mine sites, but mineralization rates can be 3 times lower on the mine site (LeFebvre and Simon, 1979). This low nitrogen supply would be reduced further by leaching due to the mine soil's high infiltration capacity (LeFebvre and Simon, 1979). Mine sites also tend to have low phosphate availability, low organic matter due to limited vegetation growth and generally dry sandy soils giving drought conditions (Kohl, 1997).

Nutrients move in the soil towards the root surface by bulk flow and diffusion (Taiz and Zeiger, 1991). Bulk flow is dependent on the flow of water through the soil towards the root. The amount carried to the plant by bulk flow is dependent on the rate of water flow and the concentration in the soil solution (Taiz and Zeiger, 1991). Diffusion is assisted by an increased gradient as the plants uptake ions.

Plants themselves may alter soil metal availability and interactions in the rhizosphere can be complex. The rhizosphere increases the acidity of the soil (thus increasing availability), and releases organic molecules (thus decreasing metal availability) (Ross, 1994b). Roots may also avoid areas of high metal concentration in the soil. Knowledge of the exact metal transfer processes between rock, soil and plant is not well developed (Ross, 1994b).

2.3 Plant Uptake of Metals

Plants are miners of the Earth's crust (Baker, 1987). Their root system extends into the soil up to depths of several meters, exposing them to varying soil conditions and metal concentrations. Plants uptake metals from the soil through their root systems, whereby they may be transported to the above-ground parts via the vascular system. Atmospheric depositions of metal can be absorbed directly by the leaf as there is no endodermis in the leaf to regulate metal diffusion (Ross, 1994b). Woodland is generally more susceptible to this manner of uptake than agricultural areas, with hairy leaves more affected than smooth ones (Ross, 1994b).

2.3.1 Mechanisms of Plant Uptake

Uptake into the plant is either passive via the apoplast, or active via the symplast (Ross and Kaye, 1994) (Figure 2.02). The apoplast pathway is mainly for the movement of water, which moves through the cell walls without crossing membranes (Taiz and Zeiger, 1991). The inner limit of the apoplast pathway is the endodermis, and for metals to be metabolised they must pass this barrier, which can only occur by active transport (Ross and Kaye, 1994). The main route for uptake of metal ions is the symplast (Raven *et al.*, 1986). Active uptake occurs at the epidermis of the root, and the ions are transported via the symplastic pathway to the xylem.

The relationship between soil and plant metal is determined by the bioavailability of the metal and the zone of metal enrichment relative to root location (Ross, 1994b). Soil metal is nearly always greater than plant metal concentration as not all of the soil metal may be bioavailable (Ross, 1994b).

2.4 Plant Response to Excess Metal

2.4.1 Metal Requirements of Plants

A model non-tolerant plant's growth response to concentrations of two metals (one essential, one non-essential) is given in Figure 2.03. Low concentrations of the essential metal limit the growth of the plant, while low concentrations of the non-essential metal do not limit the growth of the plant. At medium concentrations of the essential metal the plant enters a zone of luxury, the plant has enough of this metal so its growth is no longer limited by it. The non-essential metal has no limiting effect on the growth of the plant. At high concentrations both metals become toxic to the plant, the plant experiences stress and its growth is reduced. Thus, three zones can be identified for an essential metal; deficiency, luxury and toxicity, and for a non-essential metal ambivalence and toxicity (Macnair, 1993). The size of the zones will vary with individual metals and plant tolerance, as discussed later. The zone of luxury is often narrow (Ross and Kaye, 1994), and essential or not, metals can exert toxic effects at low concentrations (Verkleij and Schat, 1990). Zinc and copper, the metals used in this study, are essential metals for plant growth, as well as being toxic at high concentrations.

2.4.2 Plant Stress

Stress has been defined as any external factor that results in a less than optimal growth rate, or kills plant tissue (Grierson, 1999). Every organism experiences stress, although its expression alters (Larcher, 1995; Dickson and Isebrands 1991; Joshi, 1999). A plant encounters many stress events and a multitude of stressors in its life cycle (Lichtenthaler, 1996). Stress is a state in which increasing demands made upon a plant lead to a destabilisation of its functions. If the abilities of the plant to tolerate the stress are exceeded and the adaptive capacity is overworked, the result may be permanent physiological damage or death (Larcher, 1995). Stress is a significant deviation from conditions optimal for life which leads to changes at all functional levels of organisms, at low levels it may be reversible, at high levels it will be permanent (Larcher, 1995). Plants are often stressed beyond their capacity for acclimation (Lichtenthaler, 1996). Diagnostic indicators of stress can be elicited by a wide variety of stressors, so the identity of the cause of stress will not be identifiable by its symptoms (Larcher, 1995). Stress affects the entire plant, either directly from its transportation through the plant or indirectly. Stressors rarely act individually as a stressed plant will be more susceptible to other stressors (e.g. insect attack) (Larcher, 1995, Lichtenthaler, 1996).

Stressed plants can be identified by comparison with normal plants (Larcher, 1995). There are many abiotic and biotic stress factors, with abiotic factors primarily responsible for stress in marginal habitats, and biotic factors (e.g. crowding) causing stress in good habitats (Larcher, 1995). At toxic concentrations metals have specific and non specific effects. Both deficiency of an essential element and excess of a non-essential or essential element will cause a plant stress. Most often stresses do not occur singly, with other factors reversing, weakening, reinforcing or masking the response of a plant to a single stress factor (Larcher, 1995). There is generally assumed to be a cost in overcoming a stress, with plants in stress-dominated habitats having a strategy which is a compromise between yield and survival (Larcher, 1995).

Exposure to stress can occur on a daily basis, and continuous stress and strain does not necessarily mean that damage must occur as long as the intensity and duration are within the range set by the plant's resistance maxima and minima (Lichtenthaler, 1996). In order to detect stress before damage occurs, early action is required (Lichtenthaler, 1996).

The extent to which a plant's functions are affected by a stress depends on many factors.

Species, growth form, age, vigour

Climatic and edaphic conditions

Chemical nature, concentration and duration of action of the pollutant

(Larcher, 1995).

Stresses have different effects at different stages of a plants life, with germination and flowering particularly sensitive (Alam, 1999; Jones and Jones, 1989). Metal toxicity can be identified where plants show sustained injuries which are not due to other disorders, and a phytotoxic concentration of metal has accumulated in the plant (Ross and Kaye, 1994). Stress conditions and damage can be detected using methods such as rates of photosynthesis, respiration, transpiration, stomatal conductance, the concentration and ratio of pigments and stress metabolites (Lichtenthaler, 1996). Most stress factors, even if they don't directly affect photosynthesis, will affect it indirectly (Lichtenthaler, 1996). Stress affects the optical properties of the leaf. Stress contains both destructive and constructive elements and is a selection factor as well as a driving force for improved resistance and adaptive evolution (Larcher, 1995; Grierson, 1999).

2.4.3 Effects of excess metal on plants

Metals are stress factors which reduce the vigour of plants and inhibit their growth (Baker, 1987; Lanarus *et al.*, 1993). Metal toxicity is a function of:

Quantity - the bioavailable amount of a metal the plant is exposed to.

Route of exposure - via root or leaves.

Distribution of dose/exposure time.

Type and severity of injury.

Time needed to produce injury.

(Ross and Kaye, 1994).

Different metals have different toxicities. The relative toxicity of metals to non tolerant plants was assessed by Cox and Hutchinson, (1980) to be:

$Ni > Cu > Cd > Pb > Zn$

and by Ross and Kaye, (1994) to be:

$Hg > Pb > Cu > Cd > Cr > Ni > Zn$.

The mechanisms of toxicity of a metal include:

Blocking the functions of molecules (e.g. enzymes)
Displacing essential metals from biomolecules
Denaturing enzymes
Disrupting membrane integrity
Reduction in growth
Reduction in chlorophyll content
Disrupting metabolic processes

(Ross and Kaye, 1994; Devi and Prasad, 1999; Ambler *et al.*, 1970; Fernandes and Henriques, 1991, Van Assche and Clijsters, 1986; Masarovicova *et al.*, 1999).

The phytotoxic mechanisms for a given metal ion involve different biochemical pathways in different plant species and varieties (Lanarus *et al.*, 1993). A metal's phytotoxic concentration is defined as the concentration that significantly inhibits metabolic activity without death (Lanarus *et al.*, 1993).

One of the principle causes of toxicity of many metals is the disruption of enzyme activity. This may involve a number of modes of action, binding to protein sulphydryl groups, and/or substituting for essential cofactors which may cause essential ion deficiency (Meharg, 1994). Heavy metal ions at toxic levels appear to inhibit ATP synthesis, and consequently inhibit the energy metabolism in plants (Lanarus *et al.*, 1993). Metal toxicity can induce deficiency in other metals due to competition between ions at uptake (Van Assche and Clijsters, 1986). Metals can cause a growth reduction by decreasing rates of mitosis, photosynthesis (Van Assche and Clijsters, 1986), and water absorption (Atkins *et al.*, 1982). Aluminium toxicity is the most important limiting factor for crops on acidic soils (Kochian, 1995). Davies *et al.*, (1995) looked at how zinc affected *F. rubra*; there was an increased proportion of root meristematic cells which were vacuolated. Chlorophyll concentration of plants is often used to assess the impact of environmental stress (Lanarus *et al.*, 1993). Chlorophyll's biosynthesis is inhibited by metals (Lanarus *et al.*, 1993). Two enzymes in the pathway are inhibited by the metal, δ-aminolaevulinic acid deydrase and protochlorophyllide reductase (Lanarus *et al.*, 1993), with the mechanism proposed as being sulphydryl group interaction (Van Assche and Clijsters, 1986).

Plant stress caused by heavy metal contamination can affect the timing of annual growth stages of plants. Initial leaf flush and seasonal senescence could be delayed in stressed plants, resulting in a shorter growing season (Labovitz *et al.*, 1985; Schwaller and Tkach, 1985).

There has been much work carried out on the effects of single metal contamination, but in the real world this situation rarely exists. Existing data on the effects of combinations of metals are contradictory with combinations shown to have synergistic or antagonistic effects (Smilde *et al.*, 1992).

2.5 Metal Tolerance in Plants

The theory of evolution states that there is random variation of a character (e.g. growth, metal tolerance) in a population, and individuals are selected based on those which display characters most suited to that environment. Tolerance to metal concentration is such a character. Tolerance mechanisms are developed by plants to alleviate stress imposed by normally toxic concentrations of metals (Lanarus *et al.*, 1993). The tolerance of a plant determines the stress it experiences at a given metal concentration, which in turn determines its physiological response. Tolerant individuals generally exist at low frequencies in non-contaminated soils. Following contamination, or in permanently contaminated areas, they can dominate the community. The processes which determine this are discussed here, starting with the genetics of tolerance, then how tolerance affects the individual, followed by tolerance at the community level.

It is not known what determines whether a species will possess this variation in tolerance. Some species consist solely of tolerant plants, others have some populations with tolerance and some without, and other (perhaps the majority) of species do not have tolerance. Different species have different responses to metal pollution, and differing evolutionary potential (Al-Hiyaly *et al.*, 1990; Gartside and McNeilly, 1974). A population is a group of individuals from one species living in a particular area. A population of plants that differs from other populations of plants of the same species is known as an ecotype. The evolution of metal tolerant ecotypes is a prerequisite for colonisation of contaminated sites (Gibson and Risser, 1982). These ecotypes may be identical to non-tolerant ecotypes in all but tolerance to heavy metals. The frequency of tolerance in a species varies.

2.5.1 Gene Level

Metal tolerance can be proved to be genetic in origin by its propagation through the seed cycle (Macnair, 1993). Variation for a character (e.g. height, metal tolerance) in a population comes from mutation. Mutation is random in time, but not random in what it produces as it is determined by what is there before (Bradshaw, 1991). Most mutations are inviable (Bradshaw, 1991). Mutation occurs at a very low frequency, and the presence of a tolerant individual in a non-contaminated population is determined by past mutations and gene flow (Briggs and Walters, 1997). There is no bias in species that can evolve tolerance and all tolerant plants have variation for tolerance in normal populations (Baker, 1987). De novo mutation of tolerance has not been demonstrated (Turner, 1994).

There are three models (positions on a continuum of possibilities) for describing the possible genetic makeup of plants:

A single major gene, possibly with other modifying genes

Multigenic, a small number of genes with a large effect

Polygenic, a large number of genes with a small individual effect (Macnair, 1993).

A single major gene that gives tolerance is more likely to spread, and a single adaptation is often all that is required for metal tolerance to evolve in highly contaminated sites (Macnair, 1993). Further evolution would lead to more genes that improve the tolerance. Mutation of one gene to confer tolerance is more likely to occur than simultaneous multi-genic evolution. As the evolution of tolerance occurs quickly it is likely that a simple genetic system is responsible (Verkleij and Schat, 1990), although other studies have suggested a polygenic mechanism (Gartside and McNeilly, 1974). The genetic basis of tolerance may suggest mechanisms for tolerance (Macnair, 1993). For example, a polygenic system may suggest a complex tolerance mechanism, whereas a single gene would suggest that a primary physiological change needs to occur to allow the plant to tolerate contaminated conditions (Macnair, 1993).

Mutations spread through a population by gene flow. This is the spread of genes in and between populations by transport of pollen and seed. The spread of an advantageous mutation is typically limited to 1.5 metres per generation, although this will vary with the method of dispersal Levin (1988) cited in (Bradshaw, 1991). The recent and ongoing GM debate contains much contradictory evidence as to the potential for gene flow.

Some plants have evolved tolerance to more than one metal. This could arise from co-tolerance (pleiotropy) where the same genes give tolerance to more than one metal, or multiple tolerance, which involves plants having different genes each tolerant to different metals, some of which aren't in the soil of origin (Macnair, 1993). Populations can be multi-tolerant simply due to gene flow from surrounding areas where a different metal is present (Macnair, 1993). Specific genetic adaptions occur to specific metals, some of which give a low level of co-tolerance (Schat and Vooijs, 1997).

2.5.2 Individual level

A typical growth response of tolerant and non-tolerant plants to an essential metal is shown in Figure 2.04 (after Macnair, 1993). One ecotype is more tolerant than the other to high concentrations of the metal. At low concentrations the metal is limiting the growth of both ecotypes (as in Fig. 2.03). At medium concentrations they enter a zone of luxury, where they have enough of this metal so it is not limiting their growth, and can cope with its excess. The position of delimiter between these zones may be the same for both of the ecotypes. At high concentrations the metal becomes toxic to the non tolerant plants, and growth is reduced. The tolerant plants do not suffer stress at the same concentrations, and their growth is not reduced. At still higher concentrations, a level is reached where the tolerant ecotype experiences stress, and its growth is reduced. It is not clear whether tolerance arises by shifting the whole graph to the right (Figure 2.05), or if the zone of luxury is extended to the right (Fig. 2.04) (Macnair, 1993). If the metal in toxic concentrations is an essential element, many tolerant plants need a higher concentration of that element than non-tolerant plants (Verkleij and Schat, 1990), suggesting that the whole graph moves to the right (Fig. 2.05).

Tolerant individuals exist at a low frequency in some populations on non-contaminated sites. Populations vary in the frequency of tolerant individuals and in their level of susceptibility to metal pollution which may affect their ability to evolve tolerant races (Macnair, 1993). Bradshaw, (1984) found that *Lolium perenne* individuals in a normal population were 0.005% tolerant (i.e. 1/2000 plants were tolerant). Meharg and Macnair, (1992) found that 65% of *Holcus lanatus* individuals were tolerant in a normal population taken from Exeter. Gartside and McNeilly, (1974) screened normal populations of 8 species for tolerance, and found tolerance only in those also found on contaminated sites. Symeonidis *et al.*, (1985b) took seed of *Agrostis capillaris* ecotypes from various contaminated and non-contminated sites. In different populations individuals were tolerant to different metals. In one population only one individual was discovered which was tolerant to zinc, in another population individuals were tolerant to each metal tested except zinc. Not every population of a species has the same evolutionary potential, different populations may or may not be tolerant (Symeonidis *et al.*, 1985b).

It is generally accepted that there is a cost to metal tolerance (Baker, 1987, Grime, 2001). A general feature of stress tolerant ecotypes is a lower competitive ability and growth rate (Macnair *et al.*, 1993). Maximum growth rate is generally negatively correlated with the degree of resistance (Verkleij and Schat, 1990). However, there is no clear evidence of how the cost of tolerance arises (Macnair, 1993), although it may be through the tolerance mechanism requiring energy (Harrington *et al.*, 1996). A cost of tolerance may be expected, because if there was no cost, tolerance genes would be at much higher frequencies on non-contaminated sites, which is not the case (Wilson and Keddy, 1986).

In monoculture, tolerant plants have slightly less growth than non-tolerant plants (Harrington *et al.*, 1996), but in competition tolerant plant growth is much less than the non-tolerant plants (Cook *et al.*, 1971). Hickey and McNeilly, (1975) investigated the competition between tolerant and non tolerant ecotypes on a normal soil for different species. Tolerant ecotypes showed less competitive ability on normal soil for all species, with the degree of difference between ecotypes changing in each species.

Metal tolerance may not always be correlated with small growth habit or poor competitive ability, as tolerance and vigour may be controlled by separate genes (Cook *et al.*, 1971). Many mine plants may have poor competitive ability as they do not undergo selection for competitive ability as they have no competitors (Cook *et al.*, 1971; Nicholls and McNeilly, 1985). On a mine site, selection for small growth form (less competitive) may be independent of tolerance due to wind and acid exposure selecting for it (Cook *et al.*, 1971). The mechanism of tolerance may also mean that there is no apparent cost to tolerance, although this has only been found in *Holcus lanatus* on arsenic (Meharg and Macnair, 1990).

Tolerance to more than one metal can occur due to a combination of specific tolerances, or tolerance to one metal conferring tolerance to others (Schat and Vooijs, 1997). Tolerance to more than one metal not at the site is rare (Antonovics *et al.*, 1971). Tolerance in a population to a metal not present in the soil may occur due to gene flow from an area contaminated by that different metal, from seeds transported in by mine workers travelling from mine to mine (founder effects) or as a by-product of tolerance to other metals (i.e. those actually present on this site). As these tolerances are non-functioning, it might be expected that they will be lost by natural selection due to the cost of tolerance (Schat and Vooijs, 1997).

Symeonidis *et al.*, (1985a) studied tolerance in three ecotypes of *Agrostis capillaris* and found co-tolerance to metals not on the sites the plants were taken from. *Deschampsia caespitosa* is present in many toxic sites. Individuals from a site contaminated with Ni and Cu (Sudbury smelter, Ontario) were tested for tolerance, and found to be tolerant to three metals not on the site. This suggests a common physiological mechanism of tolerance to that group of metals in those plants (Cox and Hutchinson, 1980).

The mechanism of tolerance is not known for most species, and few generalisations are possible (Harrington *et al.*, 1996; Baker, 1987). The resistance of plants to metals is due to avoidance, where the plant is protected externally and tolerance where there are specific physiological mechanisms to enable plants to function normally (Baker, 1987). The same concentration of metal may enter tolerant and non-tolerant cultivars, with the metal having less of an effect on the tolerant ecotypes (Gregory and Bradshaw, 1965).

Avoidance can occur due to the existence of mycorrhiza, decreasing membrane permeability, extending the rooting system to uncontaminated areas, change in the metal binding ability of the cell wall or increasing exudation of metal chelators (Verkleij and Schat, 1990).

Tolerance is achieved by:

- production of metal binding compounds - to inactivate the metal in the cell
- alteration of metal compartmentalisation patterns - storing metals in metabolically inactive spaces
- alteration of cell metabolism - so it is not affected by the high concentrations of metals
- alteration of membrane structure - stopping excess metals entering the cell or binding them to the cell wall

(Turner, 1994). All of these mechanisms would result in metal ion homeostasis, which is fundamental to tolerance (Meharg, 1994).

The primary metal binding chemical in plants is phytochelatin. Phytochelatins are cystine rich peptides which bind non-specifically to metals in the cytoplasm (Fernandes and Henriques, 1991). The enzyme phytochelatin synthase produces phytochelatin from glutathione, the enzyme being activated by the presence of heavy metals (Dubey, 1999; Xiang and Oliver, 1999). Photochelatin is synthesised in tolerant and non tolerant plants with its concentrations similar in both, so is not wholly responsible for differential tolerance between ecotypes. However, phytochelatins may be essential components in a tolerance mechanism where tolerant plants survive the metal stress imposed and phytochelatins maintain cellular homeostasis. Davies *et al.*, (1991) showed an inhibitor of phytochelatin to have no effect on tolerance, however. Other chelators that may be involved in tolerance include malic acid (Qureshi *et al.*, 1986; Harrington *et al.*, 1996).

Intracellular compartmentalisation and precipitation of a non-active form prevents metals interfering in cellular metabolism (Fernandes and Henriques, 1991). Compartmentalisation of metals may involve chelation as well. Harrington *et al.*, (1996) and Van Steveninck *et al.*, (1987) proposed a mechanism of tolerance that involves the chelation of the metal to reduce its cellular toxicity, followed by transport to the vacuole. The metal can be stored in the vacuole indefinitely without interfering with cell function.

Plants can have an altered metabolism allowing them to function with high intracellular metal concentrations. Tolerant ecotypes show different patterns of isoenzymes to non tolerant plants. These have the same function as enzymes in non tolerant plants but their structure allows them to function in high metal concentrations (Larcher, 1995).

The only example of a clearly defined mechanism of tolerance is the tolerance of *Holcus lanatus* to arsenate, the most soluble form of As, which is chemically similar to P (Meharg and Macnair, 1990). Tolerant plants have an altered P and As uptake system (Meharg and Macnair, 1991).

2.5.3 Population level

Over areas of natural metal contamination there is often a less diverse plant community than the surrounding environment, which may or may not be distinct from the surrounding vegetation (Folkeson and Andersson, 1988). At Tideslow Rake in Derbyshire there was a continuous cover over an ore outcrop. However, there was a non-random distribution of species, with only metal tolerant species present on the ore site (Baker, 1987). Very highly contaminated sites may have much less coverage of vegetation, such as the Parys Mountain site in N. Wales.

Evolution is directional natural selection acting on random variation in a population. As far as the evolution of metal tolerance is concerned, speciation is not the outcome. Instead tolerant populations (ecotypes) on contaminated sites are the outcome. Section 2.4.1 detailed how mutations occur, so creating random variation for tolerance in populations. This section will describe how selection occurs and shapes community characteristics.

It has already been shown how tolerant plants exist in some populations at low frequencies. Some species have tolerance, some don't, some populations have tolerance, and some don't. The presence of tolerance in a species is because a tolerant mutation has arisen in that species. The presence of tolerance in a population is a factor of gene flow, past invasion, and selection pressure. Recent mutation in contemporary populations for tolerance is too rare to be considered the cause of tolerance in a population. Gene flow will come from tolerant populations on contaminated sites and will likely only be significant over very short distances. Tolerant plants may also be in a population because of seed transport, either by animal or human activity. There is no selection for tolerance on uncontaminated sites, rather, as has already been seen, there is selection against tolerance through competitive ability (Cook *et al.*, 1971; Hickey and McNeilly, 1975). All of these factors combine to mean that where tolerant plants exist on non-contaminated sites they are at low frequencies. The one exception to this found so far is for *Holcus lanatus,* which has no appreciable cost of tolerance, and the tolerant individuals are only slightly less common off mine sites than the non-tolerant ones (Meharg *et al.*, 1993). The origins of metal tolerance appear to lie in a low frequency of highly heritable variation for tolerance in the normal population (Bradshaw, 1991).

Natural selection is the driving force of evolution. It is the process whereby plants with the characteristics best suited to an environment are more likely to survive and reproduce, with their offspring retaining some or all of their traits (varies with sexual reproduction). Seeds differ from adult plants due to gene flow (Macnair *et al.*, 1993). Pollen from a non-contaminated area may fertilise plants on mine sites. The random mixing of plant characteristics by sexual reproduction means that while the adult may be tolerant, the seeds will show a variation in tolerance from non to fully tolerant (McNeilly, 1968).

The main forces of selection on mine sites are metal toxicity, other physical characteristics of mine sites (e.g. water, nutrient deficiency and coarse substrate), and competition. Metal toxicity (as well as the other physical factors on mine sites) causes stress and death in plants not adapted for it. Plants lacking competitive ability relative to their neighbours will show limited growth and reproduction, and will eventually be excluded from a population. Selection on contaminated sites takes place at radicle emergence (the first root emerging from a seed) (Cox and Hutchinson, 1980), and at later stages of growth. At germination non-tolerant plants may die, while tolerant ones live. If the non-tolerant plants survive germination, selection on contaminated sites also occurs through competition. Non-tolerant plants are stressed, so their growth is reduced. Tolerant plants are unstressed, so can grow and outcompete non-tolerant plants. Selection off contaminated sites occurs through competition, with tolerant plants being out competed for space and resources by non-tolerant plants (Cook *et al.*, 1971). The effects of competition between two individuals may be more important in mature stages than juvenile ones (Harper, 1961 cited in McNeilly, 1968). Competition from normal populations may be the main force of selection against tolerant individuals on normal soils even though the selective pressure may be small (McNeilly, 1968).

Considering one character (tolerance to a metal), the frequency of its presence can be measured in a population (Figure 2.06). This figure assumes a continuous variation, but this variation could equally be discontinuous. In a non-contaminated site there will be a normal population of plants, which may have some tolerant individuals. Tolerant plants face selection against them as they are less fit than non-tolerant plants in this environment. On contaminated ground tolerance is of great advantage, and plants tolerant enough to survive will be selected for, and the populations frequency of tolerance will resemble the "Tolerant" series on Fig. 2.06. Plants that are much less tolerant will likely not survive germination. If inadequately tolerant plants do survive they will be stressed and may be out competed on contaminated sites as more tolerant plants will survive better, and have better growth and reproduction (Antonovics *et al.*, 1971).

A population's degree of tolerance is controlled by the force of selection for tolerance, gene flow from other sites and the presence of genetic variation for the tolerance (Gibson and Risser, 1982; Macnair, 1993). Metal contamination is an intense agent of selection, although other physical and chemical agents are important (Baker *et al.*, 1986). These could include nutrient deficiencies, or lack of an available niche (Bradshaw, 1991). The effect of a pollutant on the development of tolerance in a population will be related to its concentration, availability and stability (Turner, 1994). The development of tolerance in a community that has a gradual input of pollution is different to the primary colonisation of mine spoil (Baker, 1987). Selection forces are only strong enough to result in genotypic change when the levels of contamination are at phytotoxic levels. At this point tolerant plants will be favoured over non-tolerant individuals and a shift in community composition occurs, or there is invasion of gaps by tolerant individuals. This explains the slow increase in tolerance in such communities compared with mines. There is generally a broad degree of correlation between metal levels and population tolerance, although there is often a large variation in tolerance from plants at the same site due to a very close adaptation to the microenvironment (Macnair, 1993; Gregory and Bradshaw, 1965).

The frequency of tolerant individuals in surrounding populations and their degree of tolerance interact to determine which species can colonise a contaminated site (Macnair, 1993; Turner, 1994).

It is a predominant characteristic of plants that they do not evolve tolerance. Many species may exist in the surrounding environment, and only a few on a contaminated site (Bradshaw, 1991; Al-Hiyaly *et al.*, 1990). This could be due to tolerance not being present in that local population, the species not being present when the pollution occurred (i.e. a recent arrival) or that other ecological features of the site exclude them (Bradshaw, 1991). *Deschampsia caespitosa* was on contaminated ground wherever it was present in the surrounding area, whereas *Festuca ovina* was widespread around contaminated land but was only present on very few contaminated sites (Al-Hiyaly *et al.*, 1990). This indicates that most populations of *D. casepitosa* are tolerant, while only a few populations of *F. ovina* are. Not all populations have the same evolutionary potential, so species may be metal tolerant in one area, but not in another (Symeonidis *et al.*, 1985b). Ecotypes may differ in other ways than just the tolerance under consideration (Macnair, 1993).

That tolerance has limits is shown by the sparse colonisation of very heavily contaminated sites (Turner, 1994). The exact dose of toxic metal that a species can not adapt to any further is not known, as genetic variation and the interactions of contaminants changes the pollutant's bioavailability (Turner, 1994). Variation can be exhausted under selection (genostasis) (Bradshaw, 1991). Genostasis is responsible for the limited number of species on contaminated sites because natural variation in the surrounding population is limited (Bradshaw, 1991).

There are three main scenarios for the evolution of tolerance on a contaminated site:

i. Ancient ore site with a high metal content throughout the soil profile

ii. Ancient ore site with a low metal content throughout the soil profile

iii. Recently contaminated site, possibly with ongoing contamination.

All of these could have surrounding populations with or without tolerance, and will face different selection pressures. They will be considered in the next section as simple communities of one species, with or without tolerant ecotypes, changing over time.

Figure 2.07 shows a community sequence on an ancient ore site with a high metal content throughout its profile (scenario i.). The ore site is originally bare. Surrounding non-tolerant plants are limited on the ore site by the metal content, and die at germination should they invade. If tolerant individuals are present in the surrounding population, they may invade from there, and colonise the area. If they are not present in the surrounding area the ore site will remain bare until tolerant plants invade from more remote sites, either by animal or human transport. Anthropogenic activity and interest in mining ore sites could mean that mining activity has carried tolerant seeds from mine site to mine site. Once tolerant plants are present their coverage will increase.

A community sequence on an ore site with lower metal content is shown in Figure 2.08 (Scenario ii). It is shown here with a cover of non-tolerant plants. These are stressed by the metal, but not so much that they cannot survive. Should tolerant plants be in the local population they can invade from there, if not they may invade from more remote sites as detailed in scenario i. Tolerant plants will out compete non-tolerant plants as they are not stressed by the metal, and so can achieve greater growth and gain more resources. Metal content and competitive ability combine as selection pressures, and coverage will eventually be of all tolerant plants.

Figure 2.09 shows scenario iii, an area with “normal” soils that has a contemporary contamination event. This will result in a soil profile high in metal at the surface, and decreasing further down into the profile. The community outcome on such a site will depend on the amount of metal inputted, and the prior community composition. If tolerant plants are present in the community (at a low frequency) the amount of metal inputted will determine selection pressure. At lower levels of metal there may be no advantage to tolerance, and selection will still occur against tolerance in terms of competition. At higher metal concentrations the non-tolerant plants will be stressed, and the primary agent of selection will be metal toxicity. Tolerant plants may then dominate the community. If tolerant plants are not present in the local community then their invasion could occur at any time from remote populations. If metal concentrations get too high the site may become bare before this happens. Community composition may also depend on the position of the metal in the soil. If the main concentration is near the surface (recent contamination) the plants with shallow roots will be more exposed to the metal. Plants with deeper roots will be exposed to cleaner soil, but any young plants will be exposed to the high levels.

The basis of these scenarios can be seen in results from the natural environment, which also show differences that illustrate the subtleties associated with metal tolerance. The distribution of tolerant and non tolerant plants relative to a contaminated area was studied at Drwys-y-Coed, N. Wales, the site of an old copper mine fully covered with vegetation and having an area of very high copper contamination (McNeilly, 1968). It would be expected that tolerant plants exist on the site, with non tolerant plants off the site (like in Scenario 1). This was not the case, however, with tolerant plants on non-contaminated soil on one side of the contaminated area, but not the other. This is due to gene flow. The site is in a U-shaped valley bottom and due to the valley orientation, there is a dominant wind direction (from east to west along the valley). McNeilly (1968) studied two transects, one running from across the wind, and the other running downwind (Figures 2.10 and 2.11). The crosswind transect was the most easily explained, and corresponds with Scenario 1. Only tolerant plants can exist on the mine due to the high level of metal, off the mine tolerance decreases over a short distance and the surrounding areas are non tolerant (Fig. 2.10) (McNeilly, 1968). The downwind transect shows tolerant plants off the mine site (Fig. 2.11). With the valley direction forcing a near constant wind direction, gene flow in this direction is high and means that despite selection against tolerant plants they are present on non-contaminated soil (McNeilly, 1968), as gene flow here is greater than selection against tolerance (Cook *et al.*, 1971). Most sites will show a steep cline off the contaminated site for tolerance, as gene flow will not be so directed nor selection so strong. An exception is for *H. lanatus*, which shows no steep cline as there is little cost of tolerance so selection against tolerance is weak (Meharg *et al.*, 1993).

There are no studies relating to Scenario 2(Figure 2.08), as there is little research interest in studying a stressed area waiting for invasion by tolerant plants. However, this scenario does follow the principles of tolerance, and is valid.

There are examples of the evolution of tolerance on recently contaminated areas from a copper factory, road verges, and underneath electricity pylons (Scenario iii). Prescot in Lancashire, UK, is a site of a copper rolling works with very high metal content (Wu *et al.*, 1975). Lawns have been maintained through replacement of the topsoil. Where the top soil is around ten years old metal seepage has occurred, leaving bare ground and some surviving clumps of grass. These are tolerant individuals, and areas which are 15 years since topsoil replacement show full cover (Wu *et al.*, 1975). There are only five tolerant species on the site, showing the limited number of species locally that express tolerance (Wu *et al.*, 1975).

Tolerant plants were studied alongside a road verge during the era of high levels of lead pollution from car exhausts (Atkins *et al.*, 1982), a pollution scenario on the decline due to recent advances in petrol formulation. The level of contamination would have minimal toxic effects on non-tolerant plants, but still resulted in the evolution of tolerance in *F. rubra* up to 7 metres away from the road edge. The degree of tolerance was closely related to the amount of lead available in the soil (Atkins *et al.*, 1982).

Pylons are galvanised structures, and as such deposit zinc onto the normal soils underneath them following rainfall, which acts as a selection pressure limiting the growth of non-tolerant plants. The community of plants in zinc polluted sites included tolerant as well as non-tolerant plants (Al-Hiyaly *et al.*, 1988). The presence of non-tolerant plants could be due to relatively weak selection pressure as the soil has only recently been contaminated, and the short length of time of contamination. Only a few species present in the surrounding populations showed tolerance, showing the limited variation for tolerance in the local populations (Al-Hiyaly *et al.*, 1988; Al-Hiyaly *et al.*, 1990).

It is not just the metal content that is important in determining community composition. Not all species tolerant to a pollutant may be found on contaminated land, as they may have other edaphic requirements.(Al-Hiyaly *et al.*, 1990; Baker, 1987). Tailings may lack nutrients, have high acidity (from the weathering of sulphide ores), and various physical factors (e.g. drought, exposed) (Smith and Bradshaw, 1970; LeFebvre and Simon, 1979; Nicholls and McNeilly, 1985). Some plants may be tolerant to these other factors too (Smith and Bradshaw, 1970). Thus, even though a species may be tolerant to the metal concentration at a site, it still may not be there due to a less obvious secondary character (Bradshaw, 1991). Gartside and McNeilly, (1974) showed that *Dactylis glomerata* has copper tolerance, but only existed on contaminated sites where other nutrients were not limiting. In a similar study Clark and Clark, (1981) showed that floristic richness was related to levels of lead in the soil as well as other nutrients. Vegetation coverage is seldom limited by the toxicity of the soil (Macnair, 1993). Nagy and Procotor, (1997) looked at an ultramafic site in Scotland where the plants there were tolerant but cover was only at around 10%. After the application of fertiliser there was a rapid increase in cover and individual plant size.

The identity of the species present on a contaminated site has been proposed as a means of identifying the metal present, as each metal can have a definite and distinct species association (Antonovics *et al.*, 1971). Many metal contaminated areas have a plant community with very few species (LeFebvre and Simon, 1979). Geobotany, the use of plants to indicate ores and/or metal outcrops, has a long history (Canon, 1960). The area of contaminated ground may often be recognised by the vegetation difference compared to its surrounding area, e.g. in the Congo, lack of trees (just low shrubs) can indicate Cu deposits (Antonovics *et al.*, 1971; Canon, 1960). Such obvious changes in vegetation can be located using aerial photography (Canon, 1960). In N. Greece ore bodies at the soil surface were easily located during the growth period by the reduced height of wheat plants growing there (Lanarus *et al.*, 1993). Later budding, and earlier senescence and chlorosis due to stress are other features symptomatic of metal contaminated sites (Masarovicova *et al.*, 1999; Pell and Dann, 1991; Raines and Canney, 1980).

The plants give, in effect, a 3-dimensional picture of the soil (Cannon, 1971). What may change about a contaminated area, and so what is looked for in geobotany is:

General appearance of plant cover and dominance
Composition of community
Pattern of plant distribution
Any growth deformations
Changes in the vitality of plants
Bare of vegetation

(Cannon, 1971).

When it comes to assessing the amount of metal in a soil, the soil content can be measured directly, or the level of metal in the plant can be measured. Plant sampling (i.e. chemical determination of metal concentration in the leaves) may be an improvement over soil sampling as the plant roots themselves cover a larger area than one soil sample would, and extend below ground (Canon, 1960). Foliar analysis has been suggested as a mechanism for measuring the level of metal in a soil. Although this may have the advantage of effectively measuring the level of metal in the soil over the entire range of the rooting system, it does have a number of disadvantages.

Soil properties affect metal transfer

Roots may sequester metal

No chemical/toxicant interaction is taken into account

Foliar chemistry may be affected by other factors.

Thus, plants uptake is complex and depends on many soil and plant factors (Canon, 1960), and also the plants roots may actively avoid areas of contamination (e.g. avoid the top soil where atmospheric deposition occurs). As direct foliar measurements of plant metal contamination breaks down, so the potential of remote sensing to locate such areas breaks down.

2.6 Conclusion

Total soil metal content may not directly relate to bioavailable soil metal content. Bioavailable soil metal content may not directly relate to the metal a plant may be exposed to. The amount of metal a plant is exposed to may not directly relate to it's stress response.

The use of remote sensing to detect contaminated ground relies on there being a direct relationship between soil metal content and the stress level of the plant. This relationship can break down in a number of ways. Soil metal may not have a direct relationship with the amount of metal a plant is exposed to. This is because of the numerous soil factors affecting bioavailability, which is always less than the total metal content of a soil. The bioavailable metal content of the soil does not relate necessarily with the amount of metal the plant uptakes. This is due to the root system of plants not necessarily being distributed where the metal contamination is. The response and uptake of metal by the plant does not always relate to the amount of metal in the soil. This is because of differential plant tolerance, both between and amongst species (Baker and Walker, 1990). Some plants have tolerance, most do not. Those on contaminated areas will generally be tolerant and won't show a typical stress response.

The presence of stressed plants in a community, which remote sensing depends on to locate contamination, is then a factor of the degree of metal contamination and the community composition. Generally stressed plants will only be present prior to the invasion of the area by tolerant ecotypes, until the area becomes bare due to the high levels of metals, or if metal concentrations are so high tolerant plants are stressed.

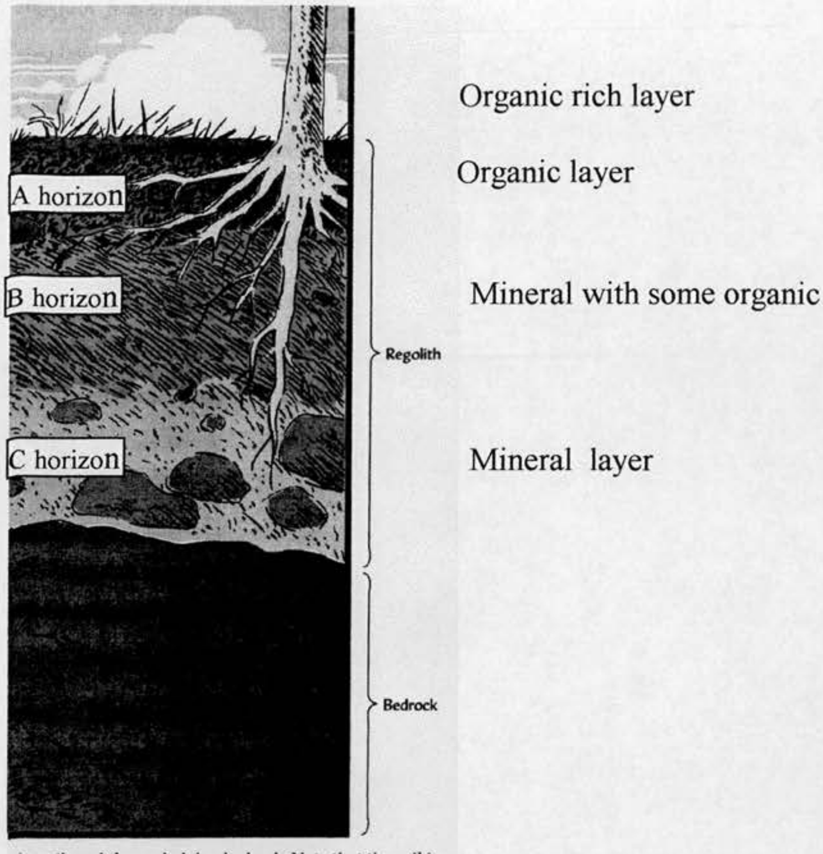

Figure 2.01. Soil profile of a typical soil. From (Brady and Weil, 1996)

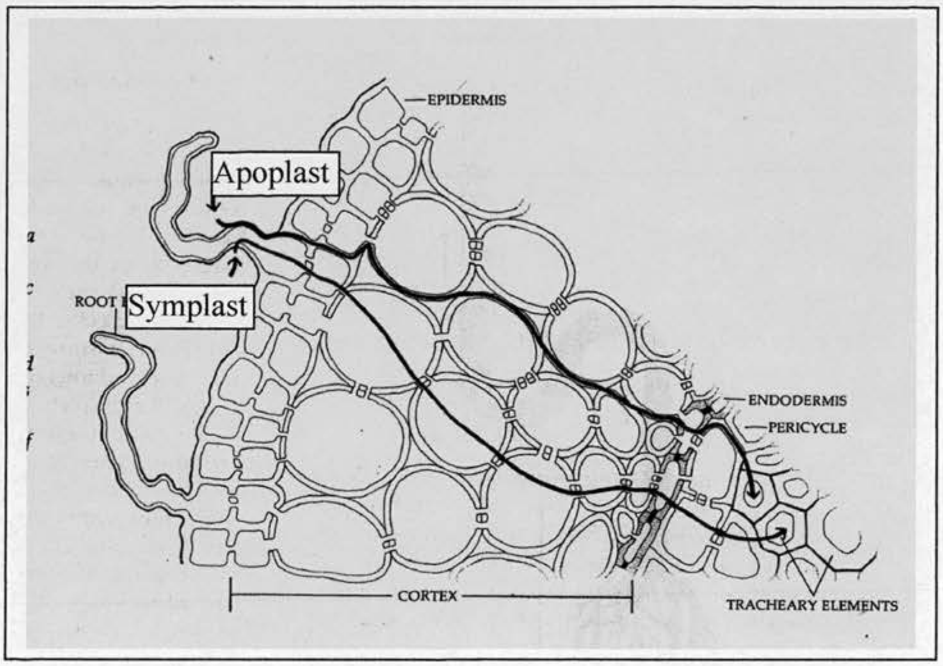

Figure 2.02. Apoplastic and Symplastic Uptake. Water mainly follows the apoplastic pathway, ions such as metals follow the symplastic pathway. From (Raven *et al.*, 1986).

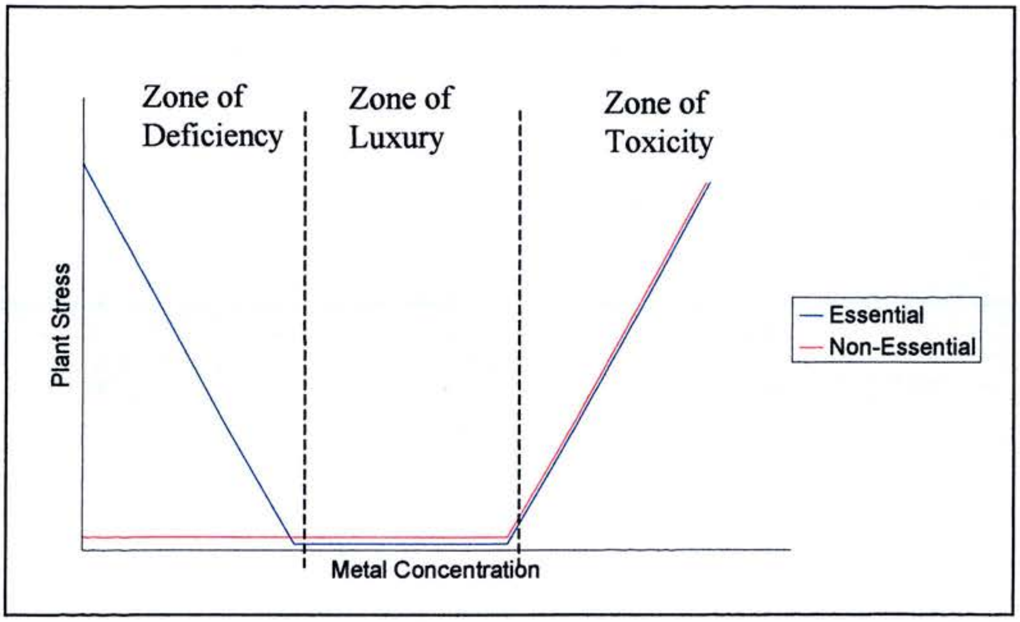

Figure 2.03. Stress response of a plant to an essential and non-essential metal. Low concentrations of the essential metal cause stress and limit growth as the plant is deficient; "Zone of Deficiency". Higher concentrations of the essential metal, and all low concentrations of the non-essential metal have no affect on stress "Zone of Luxury". Very high concentrations of both types of nutrient cause stress "Zone of Toxicity". After (Macnair, 1993).

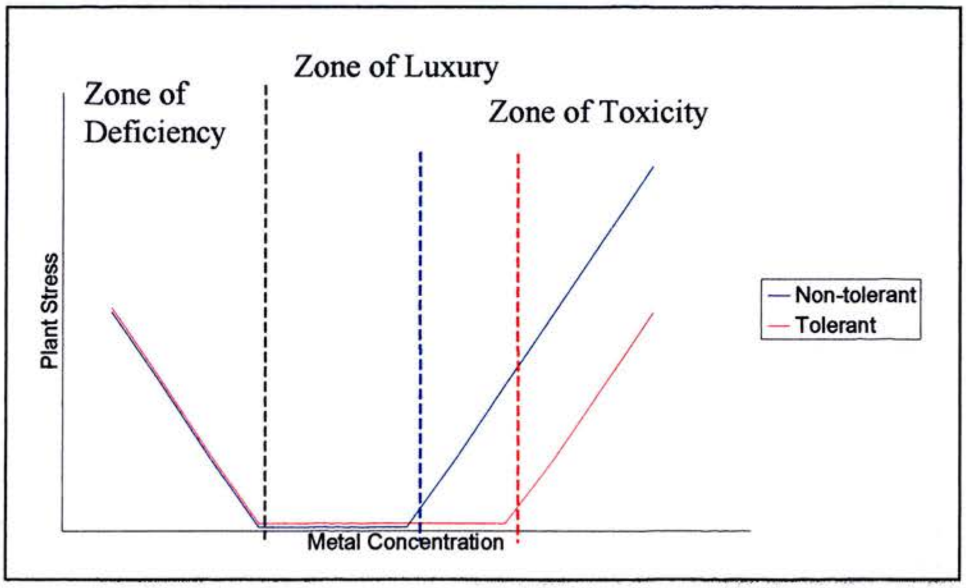

Figure 2.04. Stress response of a non-tolerant and tolerant plant to changing concentrations of an essential metal. Low concentrations of the essential metal cause stress and limit growth as the plant is deficient; "Zone of Deficiency". Higher concentrations of the essential metal have no affect on stress "Zone of Luxury". Very high concentrations in both ecotypes cause stress "Zone of Toxicity". The delimitation between the zones of luxury and toxicity vary with the tolerance of the plant (indicated by coloured zone delimiters). The tolerant plant experiences a stress effect at higher concentrations than the non-tolerant plant. After (Macnair, 1993).

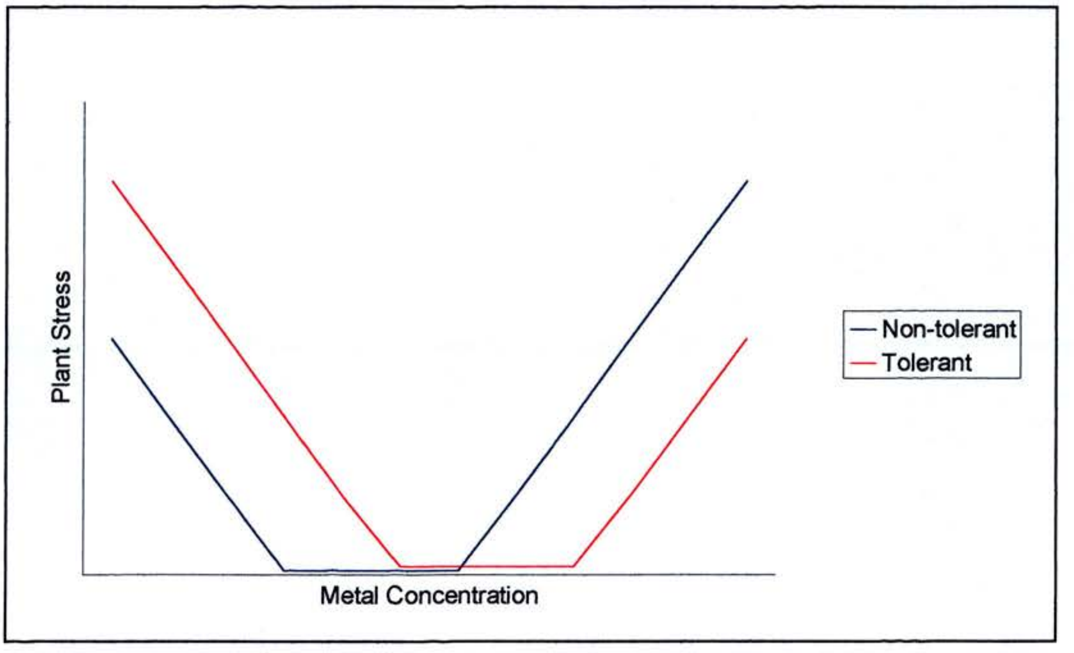

Figure 2.05. Alternative positioning of zones of deficiency, luxury and toxicity in response to changing concentrations of an essential metal in a non-tolerant and tolerant plant. Tolerant plants may be similar to non-tolerant plant except the zone of toxicity moves (Fig. 2.04) or all zones may move (this figure). After (Macnair, 1993), (Verkleij and Schat, 1990).

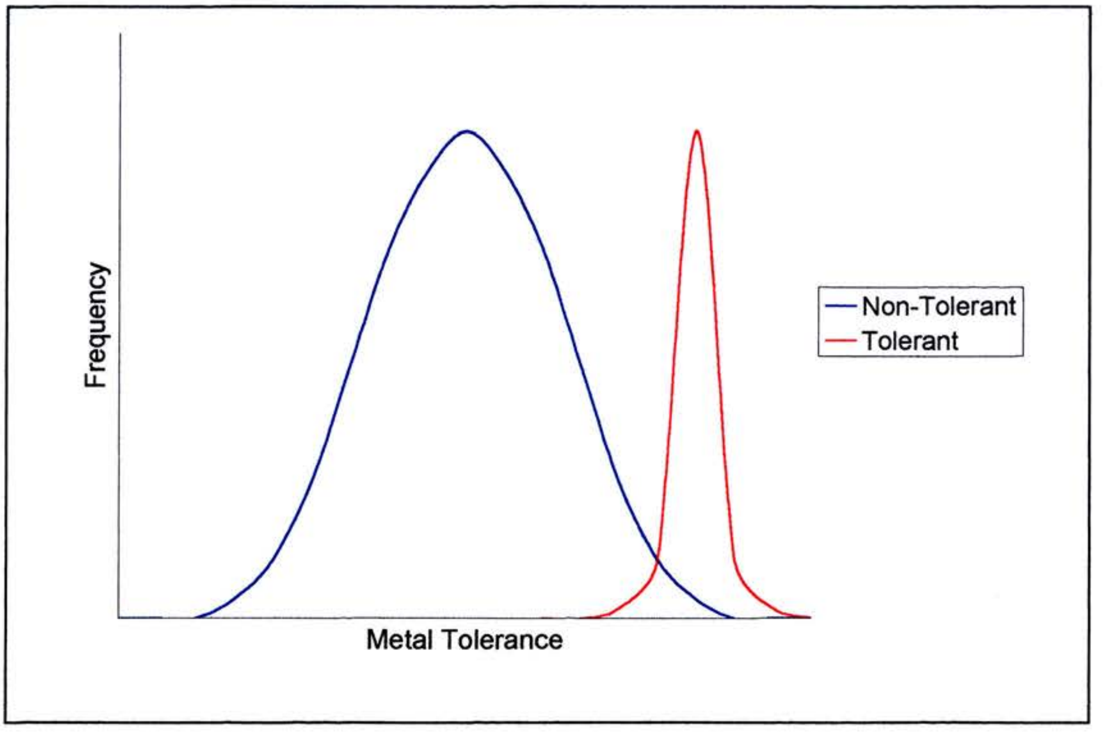

Figure 2.06. Frequency of tolerant plants in a normal and tolerant population. In a normal population a few individuals may have tolerance. In a tolerant population on a contaminated site all adults with have tolerance.

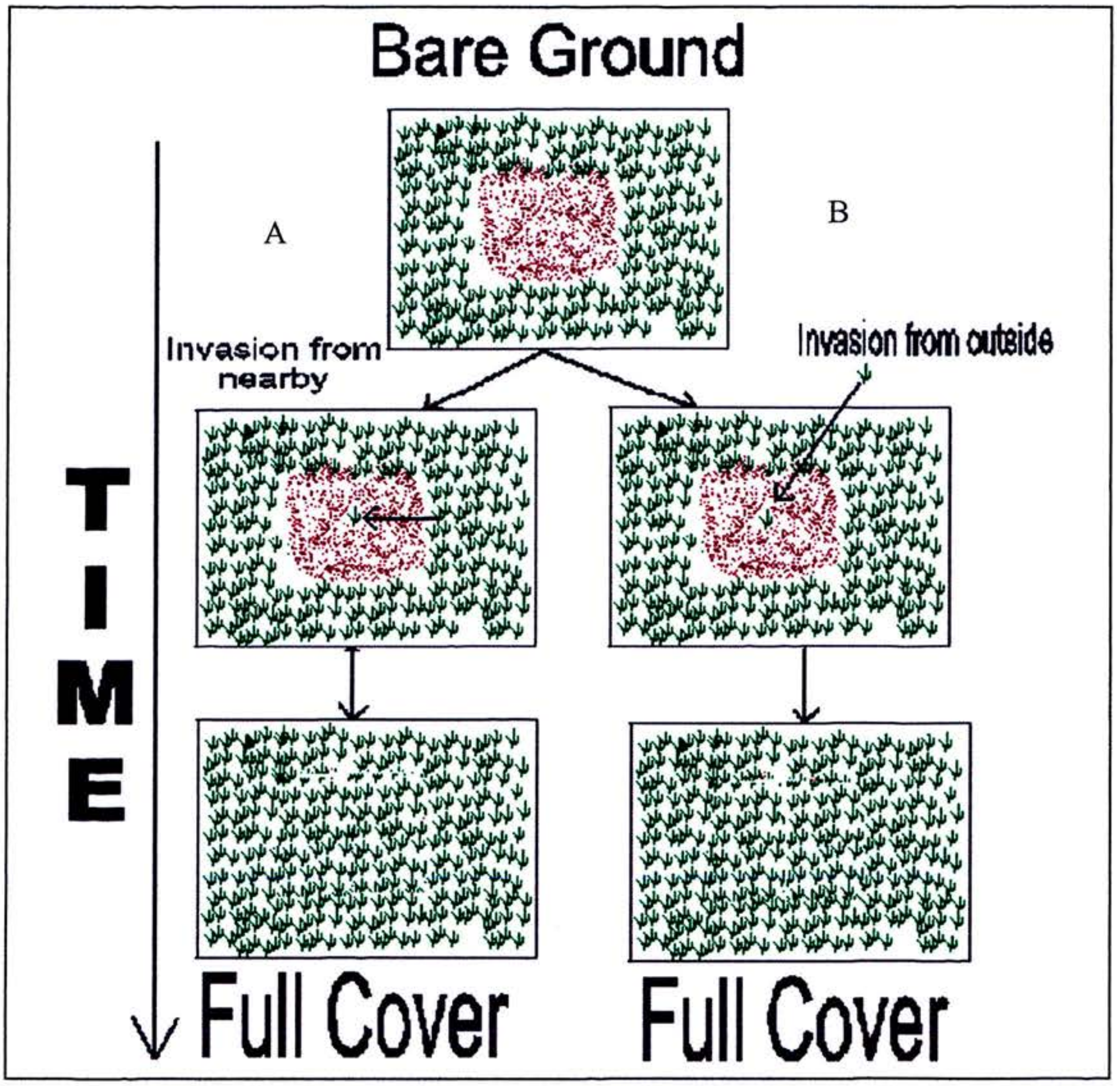

Figure 2.07. Scenario for the evolution of tolerance on an ancient ore site with high metal content (Scenario 1). Originally the site was bare due to a high metal content. With time tolerant species may invade from local (A) or distant (B) populations. These tolerant plants may continue to grow and show full unstressed cover over the contaminated site.

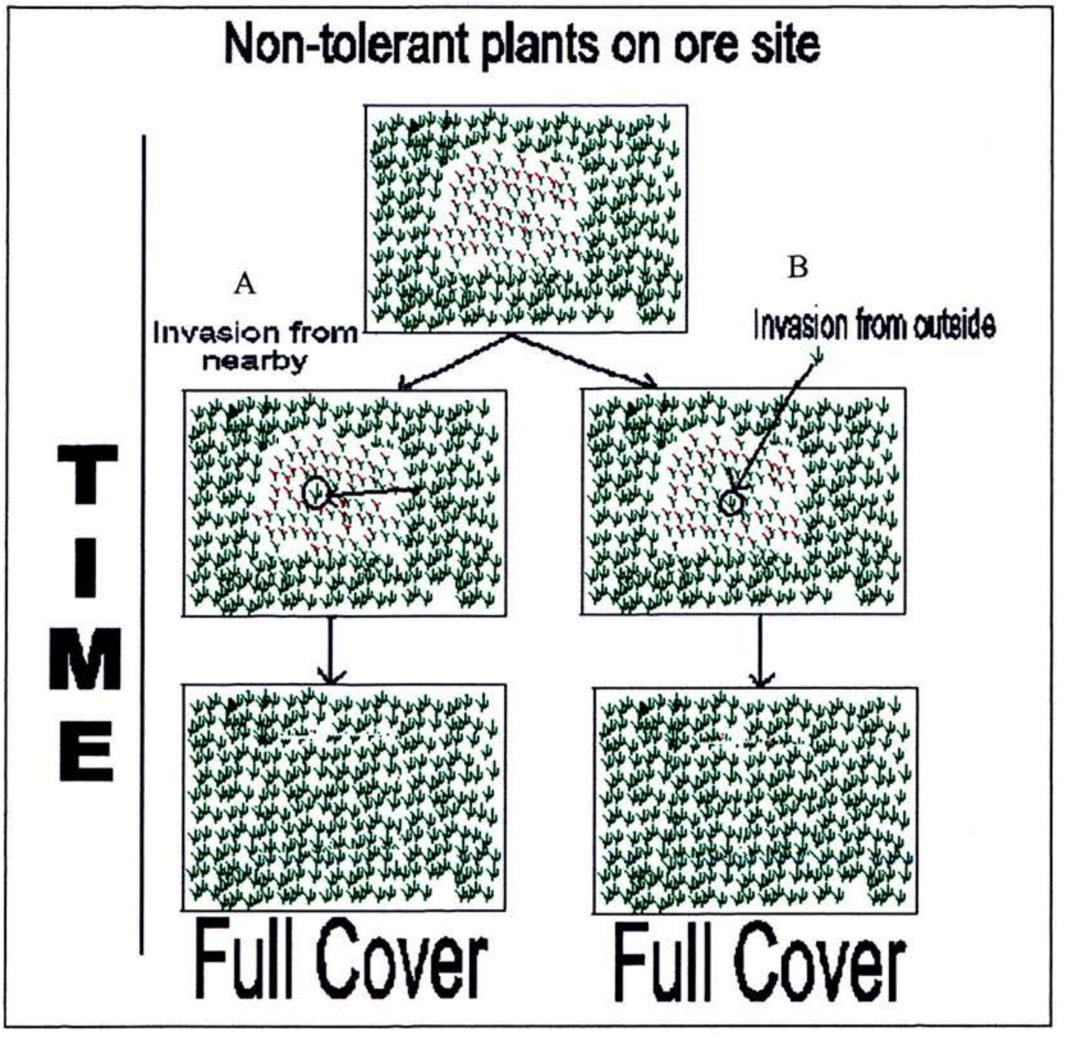

Figure 2.08. Scenario for the evolution of tolerance on an ancient ore site with low metal content (Scenario 2). Originally the site was covered with stressed non-tolerant plants due to metal content. With time tolerant species may invade from local (A) or distant (B) populations. These tolerant plants may continue to grow and show full unstressed cover over the contaminated site.

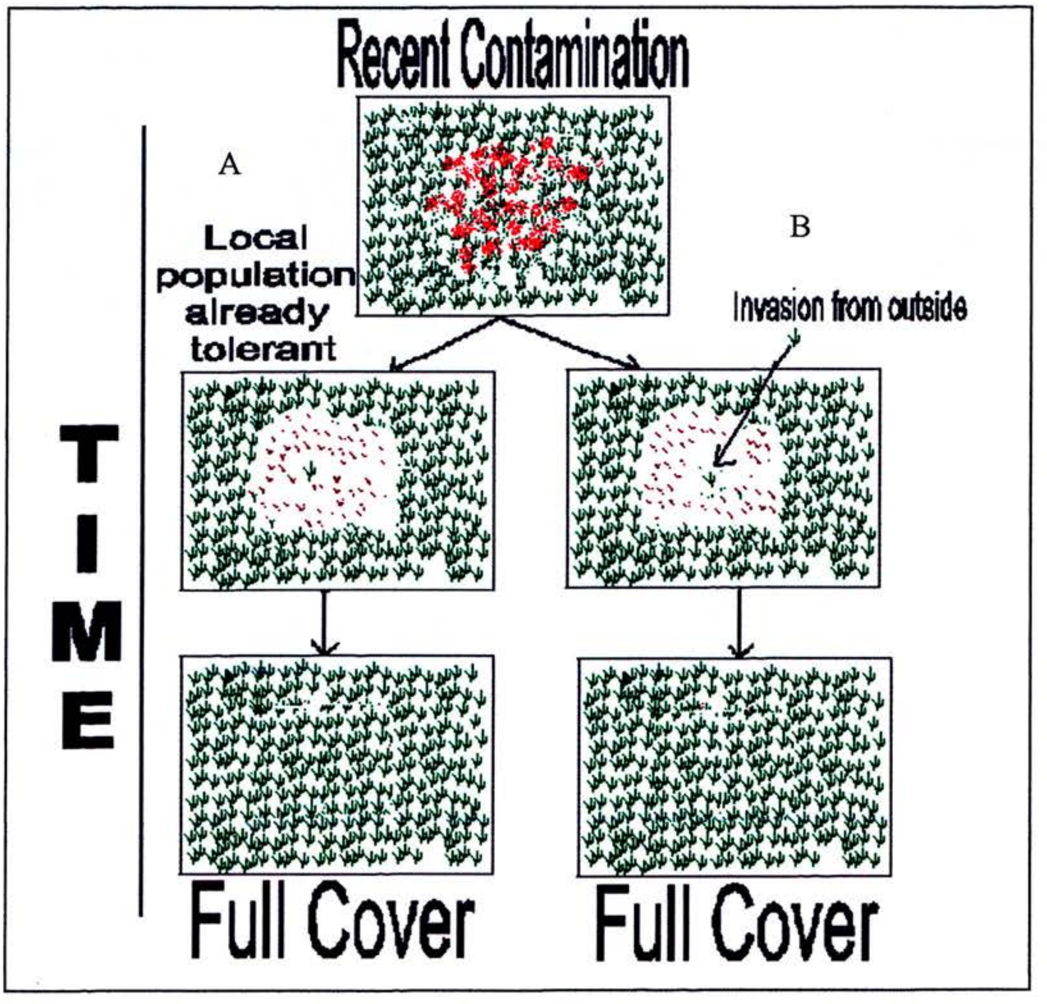

Figure 2.09. Scenario for the evolution of tolerance on a recently contaminated site (Scenario 3). Originally the site was covered with stressed non-tolerant plants due to recent metal contamination. With time these plants may die or continue to be stressed depending on metal concentrations. Tolerant species may already be present on the site but at very low levels (A) or invade from distant populations (B). These tolerant plants may continue to grow and show full unstressed cover over the contaminated site.

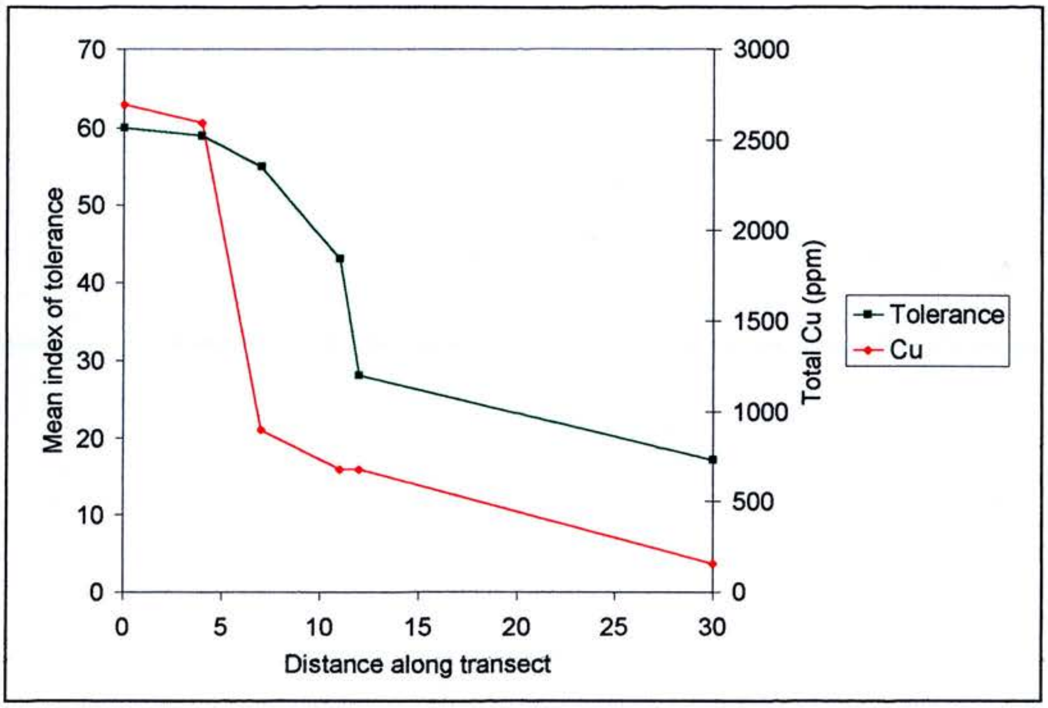

Figure 2.10. Tolerance of plants changing with the transition off a mine site - crosswind transect. At the edge of the mine site (5m along transect - Cu concentrations decreasing rapidly) the tolerance of plants decreases rapidly also. From (McNeilly, 1968).

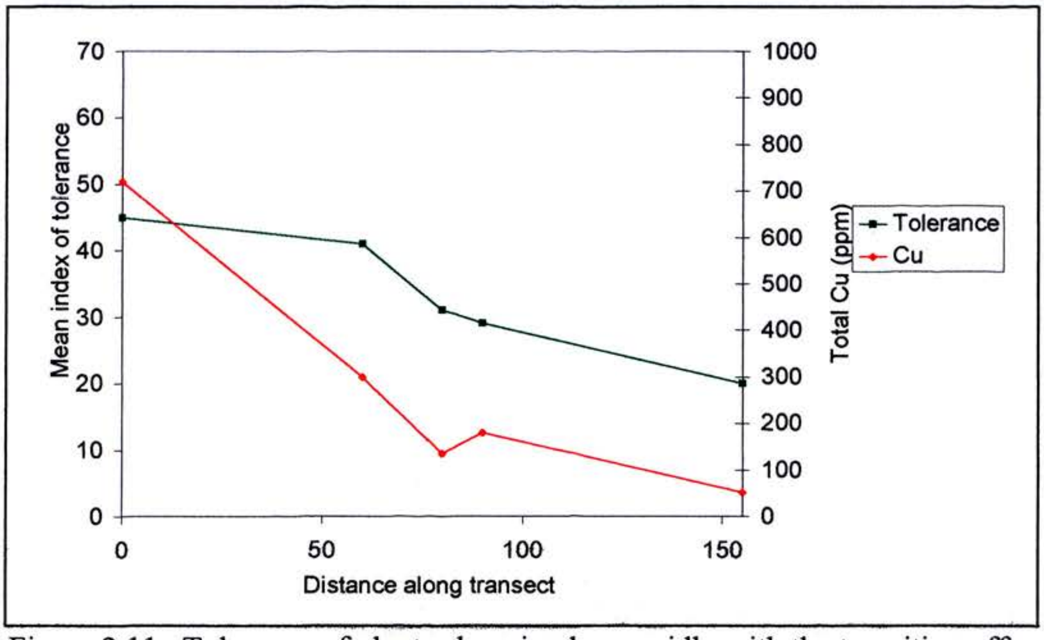

Figure 2.11. Tolerance of plants changing less rapidly with the transition off a mine site because of gene flow - downwind transect. At the edge of the mine site (at around 50m along transect) the tolerance of plants decreases a little, but not as markedly as Fig. 2.10. The difference is because of the high level of gene flow downwind off the site. After (McNeilly, 1968).

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Chapter 3: Remote Sensing of Plant Stress

3.1 Introduction

This chapter reviews the remote sensing of vegetation stress. The optical properties of leaves are reviewed first, then the impact of stress on leaf optics is discussed. Canopy optical properties follow focussing on grass canopies (i.e. leaves as the dominant component, no branches). Then remote sensing techniques for locating stress are considered, including indices and red edge position, with a concentration on those techniques used in this study. Field studies using remote sensing to locate ground contamination via vegetation stress are then reviewed. Throughout this chapter nadir sensor viewing with nadir irradiance is assumed unless otherwise stated.

3.2 Leaf optics

The fraction of light that enters a leaf is dependant on the relative angle of the leaf and leaf surface characteristics. Light is reflected, diffused and refracted by the cuticle and epidermis (Knipling, 1970). Increased surface wax levels and smoothness increases specular reflectance (Grant, 1987). Once light has entered the leaf it may be absorbed, transmitted or reflected (Figure 3.01). These three states are interrelated, being caused by the same properties of the leaf. Remote sensing concerns itself with the reflected portion of light. In order to understand what determines the proportion of light that is reflected, the mechanisms by which absorbance, transmittance and reflectance occur must be understood. These mechanisms are refraction and reflection (altered by leaf structure and water content), and absorbance (mainly affected by pigments). Light which isn't absorbed is transmitted or reflected. The path of light inside a leaf is considered first, followed by factors which determine its absorbance.

3.2.1 Refraction of light

Light is refracted upon entering a leaf, and light inside the leaf is further refracted and reflected according to the internal structure and can be considered to be diffuse. Refraction is wavelength independent and occurs mainly at the cell wall : air interface, and also at the air : water interface where there is a difference between the speed of light in the two mediums (Carter, 1991). At both interfaces there is a difference in refractive index of the two mediums (air being 1.0, cell wall being 1.4, water being 1.33 (Jacquemoud and Baret, 1990; Estes, 1983). Some refraction and reflection also occurs because of cellular structures (Gausman, 1977). Multiple refractions generally make reflectance approximately equivalent to transmission across the spectra as light has an equal chance of being refracted in any direction out of the leaf (Figure 3.02). Leaf reflectance in the near infra-red is generally high as there is little absorbance, and refraction makes reflectance more or less equivalent to transmission. Leaves which have been infiltrated with water so there are no air spaces, so no air : water or air : wall interfaces, and therefore no refraction, show decreased reflectance and increased transmittance across the entire spectrum (Figure 3.03C) (Knipling, 1970). Unpigmented plants with no water content (i.e. no pigment or water absorption) show reflectance across the spectra equivalent to NIR reflectance in normal leaves because there is little absorption and refraction still occurs (Figure 3.03A) (Knipling, 1970). Refraction is often only considered to affect the near infra-red, however as it is wavelength independent and increases the distance that a light beam takes inside the leaf refraction affects absorbance and so reflection at all wavelengths (Buschman and Nagel, 1993).

Most remote sensing work concerns itself with dicotyledonous plants, the structure of which is different from monocotyledonous plants. Monocotyledons show fewer large air spaces, and lack pallisade cells found in dicotyledons (Raven *et al.*, 1986) (Figure 3.04). Monocotyledonous plants with fewer air spaces show lower reflectance in the NIR than dicotyledonous plants (de Boer, 1993). Transmittance is generally higher than reflectance in the near infra-red because of less refraction (Baret, 1991).

3.2.2 Absorption of light

Light is absorbed in the visible region of the spectrum by the photosynthetic pigments. The main photosynthetic pigments are chlorophylls *a* and *b*, and there are also the accessory pigments, the carotenoids. All these pigments absorb light energy and use it in the anabolism of carbohydrates in photosynthesis. The wavelengths of light absorbed by photosynthetic pigments *in vitro* are shown in Figure 3.05. Extractions of the different pigments (chlorophylls *a, b,* and carotenoids) absorb light in similar overlapping regions of the spectrum. The absorption peak *in vitro* will vary with the solvent used by up to 10 nm (solvent disparity), but these give a starting point for understanding the absorption characteristics of these pigments. *In vitro* chlorophylls *a* and *b* absorb light mainly in the blue and red regions of the spectrum with the width of these absorption bands being around 25 nm. *In vivo* the absorption characteristics are different to those *in vitro* due to the solvent disparity, different pigment-protein complexes and the polymerisation of chlorophyll. Chlorophyll in the leaf is non-covalently bonded with thylakoid proteins in chloroplasts, which are destroyed by acetone extraction (Buschman and Nagel, 1993; Lichtenthaler *et al.*, 1996). Dimers of chlorophyll *a* absorb at 680 nm and 700 nm, and differing proportions of them may alter leaf absorption, and thus may alter reflectance (Collins, 1978; Lichtenthaler *et al.*, 1996).

In vivo absorption in the visible region changes from that indicated by the *in vitro* absorption spectra because of changes in the concentration of pigments and the increased travel path of a light beam in a leaf due to refraction (Gates, 1970). This is explained by the Beer-Lambert law (Equation 3.01).

Equation 3.01 $$A = \varepsilon . b . c$$

where

A = Absorbance

ε = molar absorbtivity at a wavelength

b = path length

c = concentration

Therefore as the path length that light takes increases with refraction, or as the concentration of the substance increases, absorbance at that wavelength will increase and so reflection will decrease (Figure 3.07) (Horler *et al.*, 1983; Collins, 1978). In the region of maximum molar absorbtivity (e.g. in the red region for chlorophyll) there will be little change in absorbance with concentration change as absorbance may already be at maximum . However, at wavelengths of lower molar absorbtivity absorbance can still change (e.g. for chlorophyll in the far-red region) (Gitelson and Merzlyak, 1997). These effects widen the perceived absorbance regions of pigments, convoluting them with each other.

In the near infra-red region there is a little light absorption, caused by proteins, cellulose and lignin. However, this contribution is small so transmittance and reflectance are high (Jacquemoud *et al.*, 1996). Water absorbs light energy in some regions of the middle infra-red. A decrease in water content also decreases visible reflectance (Knipling, 1970), although this is likely due to a close association of water content and chlorophyll content (Estes, 1983).

3.2.3 Reflectance of light

Looking at a typical leaf reflectance spectrum (Figure 3.06), the reflectance across all wavelengths is firstly determined by leaf structure. More interfaces increase refraction which deviates light paths, making reflectance generally equal to transmittance and broadening and deepening absorption features. Reflectance in the visible region of the spectrum is determined by photosynthetic pigment concentrations and leaf structure. Higher pigment concentrations decrease reflectance and broaden absorption features, as does higher refraction. In the near infra-red there is little absorption, and reflectance is determined mostly by the degree of refraction, with leaves with lower refraction having lower NIR reflectance and higher transmittance. Leaf structure and water content alters the amount of refraction and absorbance, and so the amount of reflectance in the middle infra-red, with increased water content resulting in more absorbance of light, and thus lower reflectance. Different leaves of the same species have different reflectance properties irrespective of their location in the canopy (Cochrane, 2000).

Leaves are non-Lambertian reflectors, meaning that reflectance is not isotropic. The angle of incoming radiation relative to the leaf surface and the surface characteristics of the leaf influences the direction of reflection of light (the Bi-directional Reflectance Distribution Function (BRDF), and so influences the reflectance perceived by the remote sensing sensor (Figure 3.08) (Goel, 1988; Salisbury *et al.*, 1987).

3.2.4 Leaf Stress

A stress may have a number of impacts on plant leaves (Table 3.01). The first response of a leaf to stress is a decrease in photosynthetic pigment concentration (in particular chlorophyll), followed by a change in leaf structure (Boochs and Kupfer, 1990). A decrease in chlorophyll concentration will cause visible reflectance to increase (Table 3.01). Given a small decrease major chlorophyll absorption regions (blue and red) would show less change in reflectance than the edges of absorption features (green, far red) (Verhoef, 2000). A large decrease in chlorophyll concentration would affect all visible wavelengths. Carotenoid concentrations are generally more stable (Prasad, 1999). Refraction may be altered by a decrease in the size of internal air spaces which will make interfaces smaller, but the number of air : cell wall interfaces may increase with the formation of micro-cavities and cell wall deterioration (Knipling, 1970). Reflectance may increase or decrease depending on the size of air spaces (smaller interfaces decrease refraction), relative frequency of air spaces (increasing refraction) and broken cell walls (increasing refraction). These effects alter reflection across the spectrum, particularly in the near infra-red (Gausman and Quisenberry, 1990) (Table 3.01). With stress, water content may also decrease leading to increased middle infra-red reflectance (Table 3.01).

Table 3.01. Leaf variables that respond to stress and their impact on reflectance across the spectrum (split into five waveband regions). Refraction is shown as increasing and decreasing as either could occur. The arrows indicate an increase or decrease in reflectance at those wavebands, and do not indicate strength of movement, "-" represents no change.

	Variable	Blue	Green	Red	NIR	MIR
↓	Pigment	↑	↑	↑	-	-
↑	Refraction	↓	↓	↓	↑	↓
↓	Refraction	↑	↑	↑	↓	↑
↓	Water	-	-	-	-	↑

After (Knipling, 1970), (Asner *et al.*, 1998), (Colwell, 1974), (Baret, 1991), (Lichtenthaler *et al.*, 1996).

These antagonistic or synergistic factors show the difficulty in relating leaf reflectance to a plant's physiological state. Stress can increase, decrease or not affect reflectance in any waveband depending on the particular stress, what it affects and the strength of that effect. Combinations of effects and stresses can have synergistic or antagonistic effects on reflectance. If a stress increases over time, and affects chlorophyll, followed by water content and then refraction (either increasing or decreasing it) the following reflectance response might be expected (Table 3.02). The red and blue regions may show no response to a change in chlorophyll concentration at first as they are at the absorption peak of chlorophyll. As chlorophyll concentration decreases further a slight increase in red reflectance may occur, and a much greater increase in green reflectance (Lichtenthaler *et al.*, 1996). If the leaf structure is affected, refraction may increase or decrease. Changing refraction has different effects in the visible and near infra-red regions. In the visible increased refraction increases absorption decreasing reflection. In the near infra-red increased refraction decreases transmittance, so increasing reflection up to a maximum equivalence with transmittance . Certain responses have a greater influence on reflectance, however; chlorophyll concentration will be the dominant cause of visible reflectance, modified by refraction. Refraction will be the dominant cause of near infra-red reflectance modified by protein, cellulose and lignin content.

In the middle infra-red water absorption will be the dominant factor affecting reflectance, modified by refraction.

Table 3.02. Response of reflectance spectra (split into 6 wavebands) to a stress event which increases in severity. Arrows indicate an increase or decrease in reflectance at those wavebands, "-" indicates no change.

	Factor	Blue	Green	Red	Far Red	NIR	MIR
T	Decrease in chlorophyll	-	↑	-	↑	-	-
I	Further decrease in chlorophyll and water	↑	↑	↑	↑	-	↑
M	Increase in Refraction	↓	↓	↓	↓	↑	↓
E	**OR**						
	Decrease in refraction	↑	↑	↑	↑	↓	↑

After (Knipling, 1970), (Asner *et al.*, 1998), (Colwell, 1974), (Baret, 1991).

3.3 Canopy Reflectance

3.3.1 Influence of vegetation component

Canopy reflectance broadly resembles leaf reflectance (low visible reflectance and high NIR) but it is not the same as if there were just multiple leaves (Jacquemoud *et al.*, 1995). The difference comes from light interacting with leaves, stems, soil and shadow and the importance of each depends on the illumination and viewing geometry and the spatial arrangement of the canopy (Baret, 1991; Knipling, 1970). Canopy reflectance is typically less than leaf reflectance, roughly 60% less in the visible, and 30% less in the infra-red (Knipling, 1970). Visible light that is transmitted through the first layer of leaves is likely to be absorbed by lower layers, and so less is reflected out of the canopy (Figure 3.09). The reduction in near infra-red reflectance is less in canopies than individual leaves as most light is transmitted through leaves, so more is reflected up from lower levels of the canopy and soil and back to the sensor (Fig. 3.09)

Leaf Area Index (LAI), the one sided leaf area per unit ground area, and leaf orientation are the most important factors determining canopy reflectance (Baret, 1991; Jacquemoud *et al.*, 1995). Increasing LAI results in decreased reflectance in the visible, and increased in the infra-red. Red reflectance decreases to a minimum at approximately LAI=2, while reflectance in the NIR region increases to a maximum at about LAI=8 (Baret, 1991). At low LAI's there is less of a response to leaf optical properties as soil reflectance becomes more important (Asner *et al.*, 1998).

Leaf orientation relative to incident radiation and the sensor affects the direction and intensity of measured reflected and transmitted light (BRDF) (Fig. 3.08). Erectophile canopies, where incident light is at an acute angle to the leaf surface, tend to scatter more radiation into lower leaf layers and have lower reflectance than planophile canopies (Jacobsen *et al.*, 1995). Leaf orientation away from the vertical increases canopy reflectance at all wavelengths because of the BDRF response, and because effective LAI is increased (Knipling, 1970). Topography also affects reflectance for the same reasons that leaf angle does. Shadows in the canopy decrease reflectance, particularly red reflectance (Colwell, 1974). An erectophile canopy such as grass will have more shadow than a planophile canopy.

Canopy reflectance will not respond to canopy variables uniquely (Baret, 1995), and canopy variables themselves are highly correlated with each other. Tucker (1977) found wet, dry, green and brown biomass, leaf water and chlorophyll content to be highly interrelated. This has the benefit that measurement of one variable could be a proxy for all (Tucker, 1977), with the drawback that environmental conditions may cause this relationship to breakdown. Thus, canopy reflectance can not be considered as unique to a set of variables (Baret, 1995) or species (Price, 1994).

3.3.2 Influence of soil background

The lower the percentage cover of vegetation, the more important soil reflectance characteristics are in determining canopy reflectance, especially in the visible region (Colwell, 1974). Soils typically show a linear increase in reflectance with wavelength, with some water absorption features in the middle infra-red (Figure 3.10). Soil reflectance is affected by surface roughness (affecting refraction), soil constituents and moisture content (affecting absorption) (Baret, 1991). Moist soils rich in organic matter have lower reflectance across the spectrum than dry sandy soils, and the higher the reflectance of a soil the greater it's effect on canopy reflectance. As more near infra-red light penetrates the canopy and is reflected back to the sensor than visible light (Figure 3.09) near infra red reflectance is more susceptible to changes in soil background (Roberts *et al.*, 1990). Furthermore, as canopy cover decreases, or if the architecture changes (becomes more vertical) exposing more soil the influence of soil on reflectance becomes more important (Table 3.03) (Goel, 1988; Roberts *et al.*, 1990). The chance of a sensor "seeing" soil also varies with view angle (Goel, 1988) (Table 3.03).

Table 3.03. Probability of directly viewing soil through a canopy varying with view angle and canopy type (After (Kimes, 1984) cited in (Goel, 1988)).

	Off Nadir viewing angle (in deg.)			
Canopy	0	25.7	51.4	77.1
Erectophile	9.70	6.40	0.82	0.00
Planophile	0.51	0.49	0.38	0.03

From (Goel, 1988)

3.3.3 Canopy Stress

Canopy reflectance will respond to stress from its effects on leaf reflectance as detailed above (Section 3.24) and through stress effects on canopy structure. In addition to effects on leaf physiology and colour, stresses may also result in a decrease in LAI, either through changes in leaf quantity or size, and hence through a change in canopy architecture (Knipling, 1970). In erectophile canopies leaf orientation will typically become more horizontal with stress and wilting so increasing effective LAI and affecting BRDF with an increase in reflectance across the spectrum. The net effect on reflectance will depend on the relative importance of a decrease in leaf size and area, and increase in the proportion of horizontal leaves. A decrease in LAI results in the sensor viewing more shadow and soil (Knipling, 1970), with there being less response to variations in leaf optical properties (Asner *et al.*, 1998). The possible responses of leaves and canopies to a stress and its impact on reflectance are shown in Table 3.04. Stress can decrease pigment concentrations, increase OR decrease refraction, decrease LAI, make erectophile canopies more horizontal, and show more soil and shadow than non-stressed canopies. These can change the degree of reflectance in different parts of the spectra in different directions (Table 3.04). Thus, the difficulties faced by remote sensing in detecting a stress response in vegetation are obvious given the different directions reflectance in any of these bands can change in response to the effects of a particular stress. Blue reflectance alone could increase due to a decrease in pigment concentration, a decrease in refraction, a decrease in LAI, an increase in the number of horizontal leaves, or it could decrease in response to an increase in refraction, or an increase in shadow. The actual response will likely be due to the net effect of all of these factors. Some factors will have dominant impacts on reflectance, and different effects of stress may occur in different strengths. Canopy factors will generally have a greater impact. These factors need to be taken into account before considering the possible plant community effects discussed in Chapter 2.

Table 3.04. Leaf and canopy variables that respond to stress and their impact on reflectance across the spectrum (split into six wavebands) for an erectophile plant. Refraction is shown as increasing and decreasing as either could occur. The arrows indicate an increase or decrease in reflectance at those wavebands, and do not represent strength of movement. "-" represents no change. Soil background is dark.

		Variable	Blue	Green	Red	Far Red	NIR	MIR
L	↓	Pigment	↑	↑	↑	↑	-	-
E	↑	Refraction	↓	↓	↓	↓	↑	↓
A	↓	Refraction	↑	↑	↑	↑	↓	↑
F	↓	Water	-	-	-	-	-	↑
C	↓	LAI	↓	↓	↓	↓	↓	↓
A N O	↑	Horizontal leaves	↑	↑	↑	↑	↑	↑
P Y	↑	Shadow	↓	↓	↓	↓	↓	↓

After Knipling, (1970); Asner *et al.*, (1998); Colwell, (1974); Baret, (1991) and using a combined PROSPECT-SAIL model (Danson, 2001).

Identification of the cause of stress lies beyond the abilities of remote sensing as the physiological responses of plants to different stresses are generally very similar. Different stresses such as nutrient toxicity or deficiency generally cause similar reflectance responses in different species (Carter, 1993). As such it may only be possible to identify a particular stress if all other stresses have been excluded (Mariotti *et al.*, 1996), an unlikely occurrence. However, there are exceptions to this. (Milton *et al.*, 1990) found that arsenic toxicity increased NIR reflectance, while selenium decreased it. These exceptions are only likely to be identified following extensive research, which would then require field studies to have knowledge of species present.

The movement of individual wavebands in different directions in response to the various leaf and canopy effects of stress does not preclude remote sensing from detecting stress. Differences in reflectance due to the various interrelating factors may be very slight, and strong responses may override them. Remote sensing also rarely uses individual wavebands alone, and generally is concerned with ratios of wavebands (vegetation indices) or characteristics of the shape of the spectrum rather than reflectance alone. Much narrower wavebands are also used which may highlight responses better (Elvidge and Chen, 1995). The complexity of the spectral responses to a stress as detailed above does make the task of stress detection hard, even before considerations are made as to the community structure of the plants that are being sensed. Various remote sensing techniques used to discern stressed vegetation from unstressed are discussed in the next section.

3.4 Remote sensing of stressed vegetation

Remote sensing studies that have focussed on the possibility of detecting stressed vegetation, or could be used to detect stressed vegetation are considered here. Both types of approach generally use different techniques to detect changes in photosynthetic pigment concentration. Other techniques may involve the changes in community structure or differences in phenology associated with contaminated areas. The two most common techniques of interpreting remote sensing data, vegetation indices and the red edge position are introduced first, followed by studies that have used them to detect vegetation stress. With many canopy parameters being interrelated (Tucker, 1977) care must be taken that remote sensing techniques establish causal links to plant stress to maximise the likelihood that any technique is applicable to other situations.

3.4.1 Vegetation indices

Vegetation indices are mathematical combinations of reflectances in different wavebands. An index therefore associates a single number to two or more spectral regions (Govaerts *et al.*, 1999). Ratioing the reflectance from two wavelengths minimises wavelength independent effects (e.g. shade, angle of illumination and leaf orientation) (Carter *et al.*, 1996; Adams *et al.*, 1999). The closer together the bands used in an index are in the spectrum then the generally better they correct for these effects. The wavebands used are generally empirically derived by correlation with the factor of interest (e.g. biomass, chlorophyll content) (Datt, 1999; Blackburn, 1998a). Two bands are generally chosen with one being sensitive to the factor of interest, and the other being insensitive (Demetriades-Shah *et al.*, 1990; Datt, 1999). Some studies then refine the choice of wavebands ensuring there is a causal relationship, while some pick wavebands based solely on an understanding of leaf optics and biology. Figure 3.11 shows the response of different possible band combinations to a stress. Example i can be considered to be a control, and an index based on these would equal 1 . Example ii shows both bands responding equally (index=1), so any index of these would be no different to the control. Example iii shows band A responding, and band B not responding so an index based on these would be different to the control (index <1), as would examples iv (index much <1; both bands respond in different directions) and v (index slightly <1; both respond in the same direction, but the difference in B is less than A) (After Morain, (1978) cited in Campbell, (1996)).

The wavebands used in indices have been chosen largely based on leaf reflectance of one or more species, and the transferability of these empirically derived indices to canopy reflectance comprised of multiple species with varied architecture is one of the major challenges to their use (Datt, 1999). Indices developed to measure canopy biomass are generally also developed to minimise interfering factors such as soil, and this is discussed more in the next section.

Indices for measuring vegetation amount.

Much of the early work in the construction of vegetation indices was concerned with detecting vegetation amount, and much of the work constructing these indices has focussed on developing indices sensitive to biomass and insensitive to soil. These indices were developed with broad band remote sensing data, and so required very obvious features of vegetation and vegetation amount. The red and near infra-red regions offer the most obvious spectral features of vegetation which respond to amount, so these bands were used in indices. Use of wavebands close to each other minimises wavelength independent effects, but this does not account for the influence of soil which shows an increase in reflectance between the red and near infra red (Section 3.3.2). Soil will mainly affect these indices at low vegetation cover because NIR reflectance shows a greater response to soil brightness than red (Huete *et al.*, 1985) (Section 3.2.3). Different soils have different reflectance slopes and the soil reflectance slope will vary with soil constituents and wetness (Demetriades-Shah *et al.*, 1990).

The soil line is comprised of the red and near infra-red reflectances of different soils. These form a line with a slope of 1 in red : near infra-red spectral space (Figure

3.12). Vegetation indices can be described based on their relationship with this line and categorised into ratio based and orthogonal indices, and a hybrid of the two (Elvidge and Chen, 1995). Isolines of equal greenness as measured with the Normalised Difference Vegetation Index (NDVI) (and other ratio based indices) converge with the soil line at the origin (Fig. 3.12A). This is because for a given greenness the ratio between red and near infra-red is the same. Greener vegetation will have higher NIR reflectance relative to red, and so the slope of vegetation of that greenness is steeper. For the Perpendicular Vegetation Index (PVI) (an orthogonal index) the greenness lines are parallel to the soil line (Fig. 3.12B) (Bannari *et al.*, 1995). The PVI measures greenness as the orthogonal distance in spectral space from the soil line, a point further away is greener. The Soil Adjusted Vegetation Index (SAVI) (a hybrid type) modifies NDVI to use an adjustment factor (L) to account for the effect of soil reflectance on red and near infra-red reflectance. As in ratio based indices lines of equal greenness converge, but in this hybrid they do not converge at the origin (Fig. 3.12C).

Many indices have been developed to alleviate the influence of different soil backgrounds, and the number of suggested indices indicates the scale of the problem. It has been suggested that different indices should be used where there are different backgrounds (Todd *et al.*, 1998), although this just shifts the problem to one of identification of different backgrounds. This technique of orientating isolines of the factor of interest (e.g. greenness in the PVI) so they are orthogonal to the factor desired to be measured (greenness) and parallel to the disturbing factor to be controlled for (e.g. soil) will be a compromise as these factors will not always be orthogonal (Govaerts *et al.*, 1999). Concentrating on minimising the sensitivity of indices to one factor (e.g. soil) may also mean that indices become sensitive to other extraneous factors, or insensitive to the factor of interest (Gemmell and McDonald, 2000).

The NDVI is a widely used index that has been proposed for measuring vegetation amount and quality. However, it has been found to be sensitive to soil background (Huete, 1988), not particularly accurate at low covers (Purevdorj *et al.*, 1998) and can confuse a decrease in chlorophyll content with low cover (Demetriades-Shah *et al.*, 1990). Different combinations of chlorophyll and LAI give the same index value (Railyan and Korobov, 1993) and it can be a weak estimator of chlorophyll (Vogelmann *et al.*, 1993). It is included in this study because of it's extensive use. SAVI (Huete, 1988) was one of the first indices to try to correct for soil background effects, and it has been modified further (e.g. as MSAVI and TSAVI to name just two). It is included here to investigate whether it offers an improvement over NDVI.

With the increase in availability of narrow band spectral data there is an increasing awareness that other spectral features may be better suited to identifying vegetation amount, such as the shape of the spectral reflectance curve. Vegetation indices may provide the best solution for broad band data, but for narrow band data other features of the spectrum may provide a better response to the desired variable (Elvidge and Chen, 1995). Appropriate narrow band indices are considered in the next sections.

Chlorophyll concentration

The best wavelengths responding to chlorophyll concentration are not necessarily those where chlorophyll absorbs maximally. These bands are responsive at low chlorophyll concentrations, but do not respond to higher concentrations as reflectance becomes saturated at low pigment concentrations (Buschman and Nagel, 1993; Gitelson *et al.*, 1996) (Section 3.12). The edges of the absorbance features generally show the most sensitive response to changing chlorophyll concentration (e.g. the far red (Carter and Miller, 1994) and green (Gitelson *et al.*, 1996) regions). This is due to the narrowing of the absorbance spectra with decreased pigment concentration (Fig. 3.07) while regions of maximal absorption show no response.

Blackburn, (1998b) developed indices for assessing changes in individual pigment concentrations (Chlorophylls *a*, *b* and carotenoids; Table 3.05). The starting point for choice of wavebands was work defining wavebands which minimise convolution and have the closest relationship between reflectance and pigment concentration (Chappelle *et al.*, 1992). These were formed into indices using a waveband in the near infra-red to control for the effects of refraction. These indices were then optimised by changing the visible wavebands used until the index showed the best relationship with pigment concentration. There were good relationships between the indices and chlorophylls *a* and *b*, but not for the carotenoids. These were tested on stacked senescent tree leaves (Blackburn, 1999) to better simulate canopy conditions and to cover a wider range of pigment concentrations. Again the indices were better related with chlorophylls *a* and *b* than the carotenoids.

Table 3.05. Vegetation indices used in remote sensing of stress.

Author	Abbreviation	Name of Index	Formulae	Application
(Blackburn, 1998b)	PSSRa	Pigment Specific Simple Ratio for chlorophyll a	800/675	chlorophyll a
	PSSRb	PSSR for chlorophyll b	800/650	chlorophyll b
	ref. PSSRa	reformed PSSRa	800/680	chlorophyll a
	ref. PSSRb	reformed PSSRb	800/635	chlorophyll b
	PSSRc	PSSR for carotenoids	800/500	carotenoid
	PSNDa	Pigment Specific Normalised Difference for chlorophyll a	$\frac{800-680}{800+680}$	chlorophyll a
	PSNDb	PSND for chlorophyll b	$\frac{800-635}{800+635}$	chlorophyll b
(Carter and Miller, 1994)		(none)	695/760	stress
(Malthus *et al.*, 1995)		(none)	425/470	stress
			446/477	stress
			541/836	stress
			818/538	stress
			818/713	stress
(Penuelas *et al.*, 1994)	WBI	Water Based Index	970/900	water stress
	PRI	Physiological Reflectance Index	$\frac{550-530}{550+530}$	plant physiology
	NPCI	Normalise Pigments Chlorophyll ratio Index	$\frac{680-430}{680+430}$	

Penuelas *et al.*, (1994) developed the PRI, NPCI and WBI (Table 3.05) using wavebands chosen based on a prior understanding of pigment absorption characteristics. These successfully used wavebands in the green and far red to measure pigment concentration (PRI and NPCI), and wavebands in the near infra-red to measure water content (WBI). All were validated using leaf reflectance.

Malthus *et al.*, (1995) chose indices for measuring chlorophyll content based on significant correlation coefficients between indices (all x/y) of all wavelengths against chlorophyll concentration (Table 3.05). Chlorophyll concentration was varied by the addition of herbicide. Chosen indices were also tested for sensitivity to changing background reflectance, with 818/538 and 818/713 showing the least response to soil and a high sensitivity to chlorophyll.

Carter and Miller, (1994) developed indices for detecting stress having measured reflectance at 420, 600, 670, 694 and 760 nm from herbicide stressed soybean plants (Table 3.05). These were combined into ratios of which 694/760 showed the best response to stress. Reflectance at 694 nm showed a good relationship with chlorosis being at the absorption edge of chlorophyll, while the near infra-red band controlled wavelength independent factors (Carter and Miller, 1994).

3.4.2 Red Edge Position (REP)

The red edge is the region on the reflectance spectrum between high absorbance by pigments in the red region and high reflectance in the NIR region due to internal refraction (Fig. 3.07). Whilst a NIR:R ratio index effectively measures the height of the red edge, another component of this part of the spectrum that has been frequently used is the Red Edge Position (REP), which is generally determined from the peak of the first derivative of reflectance in the region 680 - 750 nm. Using derivatives ignores non vegetative components of the view, including the effect of illumination, view angle and soil (Boochs and Kupfer, 1990; Curran *et al.*, 1991; Buschman and Nagel, 1993). The red edge is a complex region, comprised of several underlying components, and it has been suggested that it cannot be simplified to just one point (Railyan and Korobov, 1993). Most studies just consider the REP, however. In addition, the first derivative curve is not smooth, whether through noise or subtle effects of canopy factors.

The behaviour of the red edge is not fully explained (Railyan and Korobov, 1993). It is generally considered to be a function of chlorophyll absorbance in the red, and NIR reflectance (Horler *et al.*, 1983). Some authors consider only chlorophyll content to be important with the location of the red edge position being determined by the degree of absorbance by chlorophyll (Pinar and Curran, 1996). The higher the chlorophyll content the deeper and broader the absorption spectrum, and so the lower reflectance at longer wavelengths, moving the red edge to longer wavelengths (Section 3.1.2). With stress the REP will move to shorter wavelengths with a less deep and broad absorption feature in the red due to a decreased chlorophyll content (Collins, 1978).

The red edge is not necessarily a smooth transition between absorbance in the red and reflectance in the near infra-red. There may be two or three peaks on the first derivative in the red edge region that may vary in importance, and so move the REP (Horler *et al.*, 1983; Filella and Penuelas, 1994). As few studies mention the width of smoothing filters frequently applied to data before REP is determined, it is not possible to state whether the first derivative of red edge generally has these features; studies may not mention them because they are non-existent or because the degree of smoothing applied was too severe. Where multiple peaks on the first derivative of red edge have been identified the shorter wavelength peak is accepted to be related to chlorophyll concentration with longer wavelength peaks being related to chlorophyll content and LAI (Boochs and Kupfer, 1990, Gitelson, 1996). The dominance of either peak determines the REP.

There is little correlation between leaf and canopy red edge position (Demetriades-Shah *et al.*, 1990). Leaf REP can vary from 690 to 730 nm, while canopy REP is more stable, being around 720 nm in most cases (Demetriades-Shah *et al.*, 1990; Jago *et al.*, 1999). REP is highly dependent on changes in the geometric structure of the canopy, and changes in its structure (e.g. from wind) may be a major influence on its position (Vanderbilt *et al.*, 1988). Stress responses may not be as robust as hoped for. Milton *et al.* (1990) measured the red edge response in response to selenium and arsenic - with arsenic the red edge moved to shorter wavelengths as expected, however with selenium stress the red edge moved to longer wavelengths.

REP is also sensitive to LAI, with REP moving to longer wavelengths with increased LAI up to a LAI of approximately 8 (very high) (Danson and Plummer, 1995; Horler *et al.*, 1983). LAI thus complicates the relationship between REP and chlorophyll concentration (Verhoef, 2000). In monocotyledons stress may decrease chlorophyll content (moving REP to shorter wavelengths) but increase effective LAI by the leaves becoming more horizontal which would move the REP to longer wavelengths. Thus, with REP responding to a number of variables, one (such as chlorophyll content) cannot be retrieved accurately unless the others are known and are constant (Verhoef, 2000).

3.5 Stress Studies

There have been a number of field studies researching the ability of remote sensing to detect heavy metal contamination via vegetation stress, which are generally extensions of Geobotany. Geobotany is the detection of metal ore (or other geological features) by changes in vegetation on a particular site. Historically, geobotany has concerned itself with associating metal tolerant plant species and plant stress characteristics with ore-rich sites. It has recorded uses as long ago as in ancient Greece, through the medieval period (Agricola cited in Ustin *et al.*, (1997)) and into modern times (Russian geological expeditions always had botanists on them (Cannon, 1971)).

There are a number of vegetation changes (biomarkers) that are looked for in ground-based studies which have been investigated for use by remote sensing geobotanical studies. The analysis of the distribution of species relates the occurrence of tolerant plant species with their known metal associations. Different metals and metal combinations (e.g. serpentine soils) have tolerant species that will only be found there. This has been used in the Congo where copper sites are associated with low level herbaceous species compared to the surrounding forest (Canon, 1960). Sites will often also show a simplified (i.e. less species as only a few can tolerate the high metal levels), less dense (i.e. lower growth from energy used by tolerance mechanisms and a generally poorer soil on ore sites) community than surrounding areas. Remote sensing may be able to detect this change in vegetation density using indices of vegetation amount (Paradella *et al.*, 1994; Cibula and Lucas, 1990; Filho, 1984).

Metal rich soils can also cause stress in non-tolerant plants. Remote sensing may be used to look for direct or indirect consequences of stress (Canney *et al.*, 1979). A reduction in chlorophyll concentration has been researched by many remote sensing studies using indices and red edge position, with success at the leaf and canopy scale, although the two do not always relate (Bethel *et al.*, 2000). A change in index values or shift of the red edge position can discriminate between chlorophyll concentrations, but may be convoluted by changes in LAI (Ustin *et al.*, 1997) or other complications in the natural environment (Yang *et al.*, 1998). Greater success in differentiating between contaminated and clean sites based on pigment concentration has come from the observation of senescence. Many studies attempting to find techniques for locating contaminated ground have noted the shortening of the growth season in stressed vegetation. Metal contaminated sites have showed delayed appearance of leaves (around 7 days), and earlier senescence (Bell and Labovitz, 1985). Premature senescence and its associated large decrease in chlorophyll content is more easily identified than small stress-induced changes in pigment concentration. The time of observation (ideally frequent over the end of the season) will thus be vital to its detection (Saraf and Cracknell, 1989). The best chance of locating stress will be where the species and its spectral responses to different stresses are known (Nutter *et al.*, 2000) and with a high frequency of imaging (Pearson *et al.*, 1994).

3.6 Conclusions

Chapter 2 explained how different plants have different tolerances to metal contamination, and how plants may not respond directly to soil metal content. Very few remote sensing studies have considered tolerance. Carter *et al.* (1992) studied ozone tolerant and sensitive individuals of loblolly pine, and found they differed in response according to their tolerance. Labovitz *et al.* (1985) considered the different responses of different species to metal stress. Other studies have not considered tolerance. This does not seem important as many studies show a stress response with stress contamination where it has been looked for, but as negative results are much less likely to be published this is not necessarily indicative of all situations (Callaham *et al.*, 1998).

For remote sensing to detect contaminated ground via vegetation stress it should show a greater reflectance response (e.g. change in REP or index) with higher levels of stress. Ideally these responses would be unique to particular stresses, but given the number of stresses and similarities in plant physiological and morphological responses to stress this is not likely. Stress indices should be unique to stress, and not be confused with other canopy features (e.g. LAI). As has been shown here stress responses on aspects of canopy or leaf reflectance may be antagonistic or synergistic, varying with strength in different situations.

None of these techniques are able to detect the cause of vegetation stress (Saraf *et al.*, 1989). Metal content is not the only factor changing over a site; other soil variables change making the environment heterogeneous (Wagner and Howarth, 1989). This factor, combined with different plants having varied responses to metal contamination, will make the spectral characteristics of a contaminated site heterogenous. All techniques also rely on comparisons with a control area (Labovitz *et al.*, 1985); ideally remote sensing would be a standalone technique (Birnie and Francica, 1981). The end user of remote sensing attempting to detect stress may have to accept that the best it may offer is as a method of preliminary study indicating sites where direct metal analysis should be carried out (Schwaller and Tkach, 1985), with an understanding that false negative error is likely because of tolerance. False positive results will occur too as different stresses give the same spectral response (Carter, 1993).

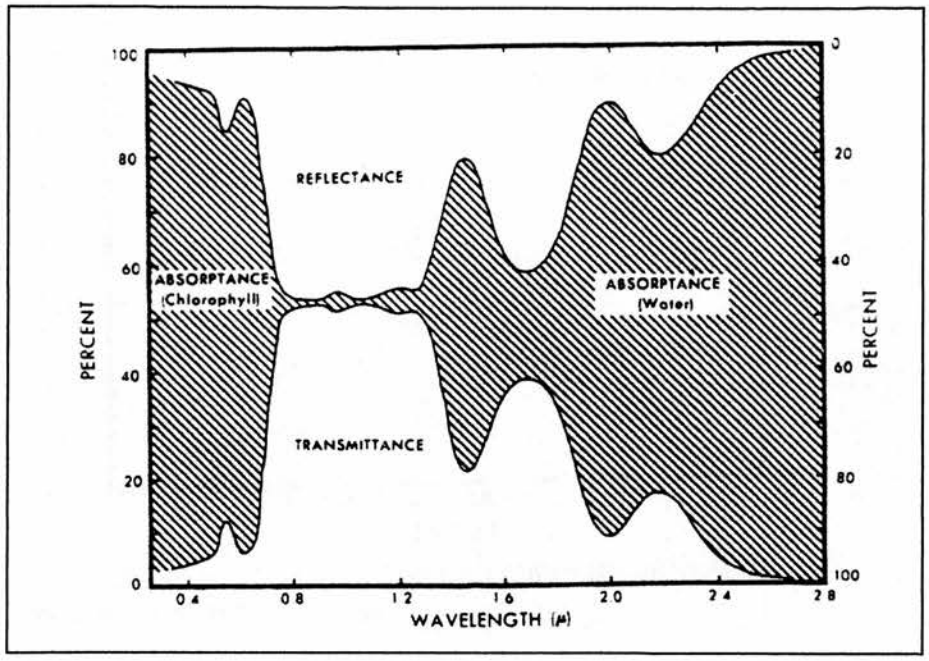


Figure 3.01. Reflectance, absorbance and transmittance spectra of a typical plant leaf. From Knipling (1970)

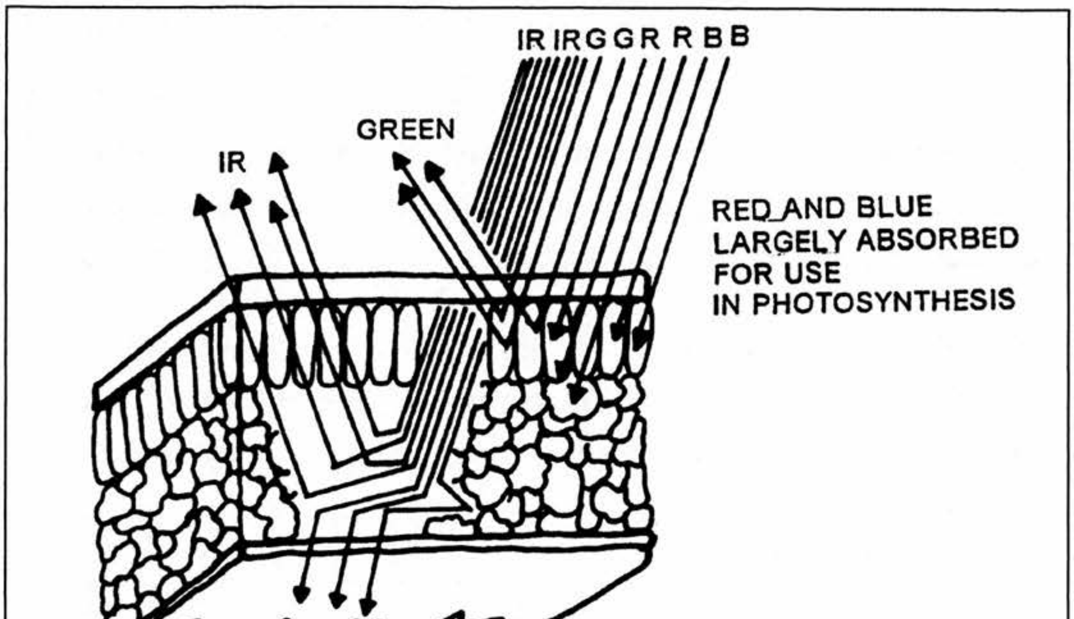
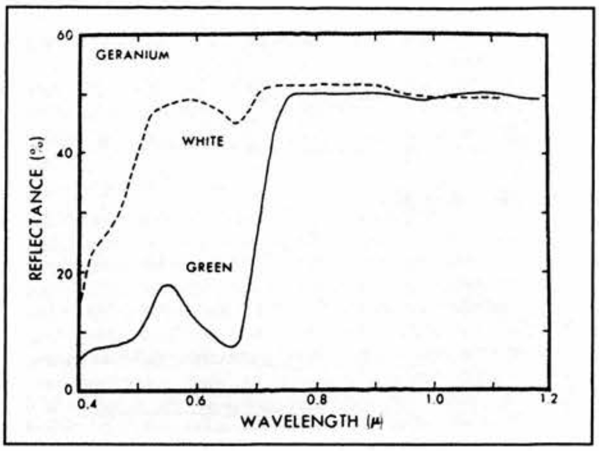
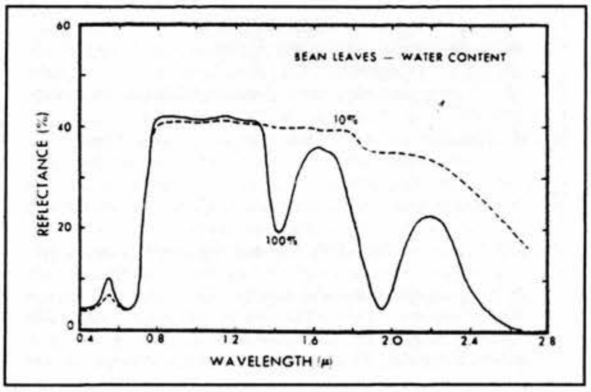


Figure 3.02. Interaction of light of different wavelengths with a leaf. Red and blue ("R" and "B") are mainly absorbed, some green ("G") is absorbed and some is reflected, near infra-red ("IR") is refracted and transmitted or reflected. From Campbell (1996).

A



B



C

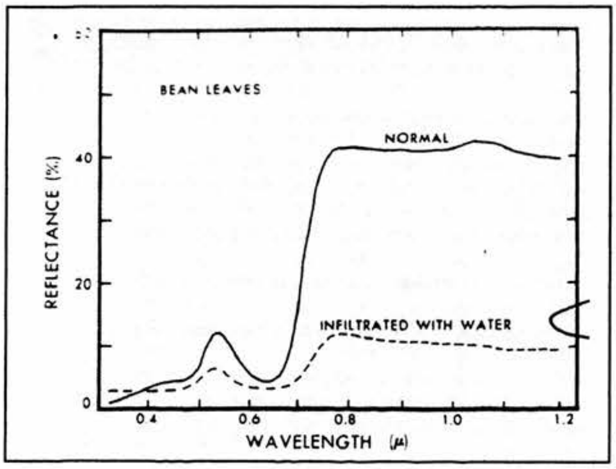
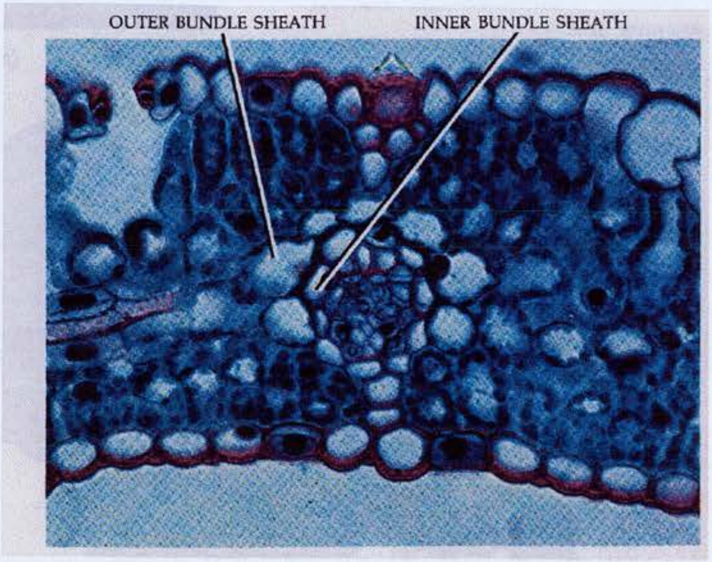


Figure 3.03. Influence on leaf reflectance of absorption and refraction. "A" shows unpigmented leaves ("White") compared to normal leaves ("Green"), with no pigment absorbance in the visible reflection is much higher. "B" shows the affect of water absorbance, with less water ("10%") reflection in the middle infra-red is higher. "C" shows the affect of much decreased refraction ("Infiltrated with water") compared to normal leaves. Reflectance across the entire spectrum is lower with lower refraction. From Knipling (1970)

A



B

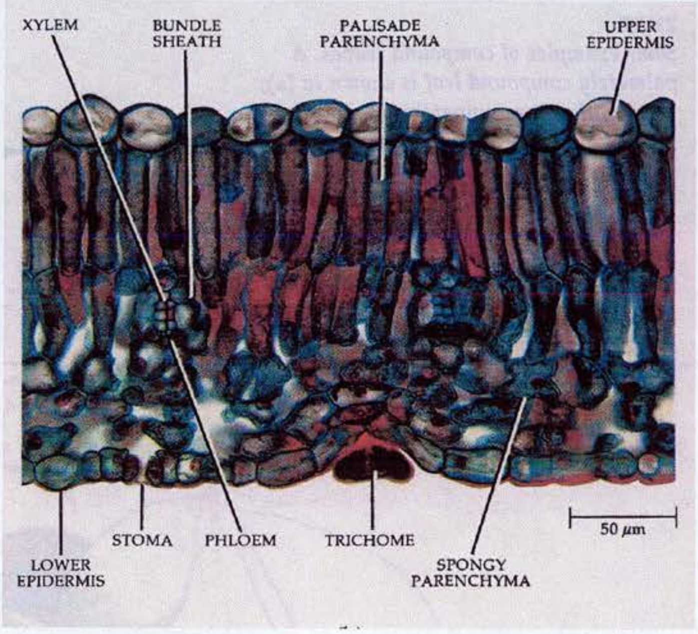


Figure 3.04. Monocotyledon (monocot; "A") and dicotyledon (dicot; "B") leaf structure. Monocots typically have less organised internal structure, less air spaces and so less refraction than dicots (de Boer, 1993).
From Raven *et al.*(1986).

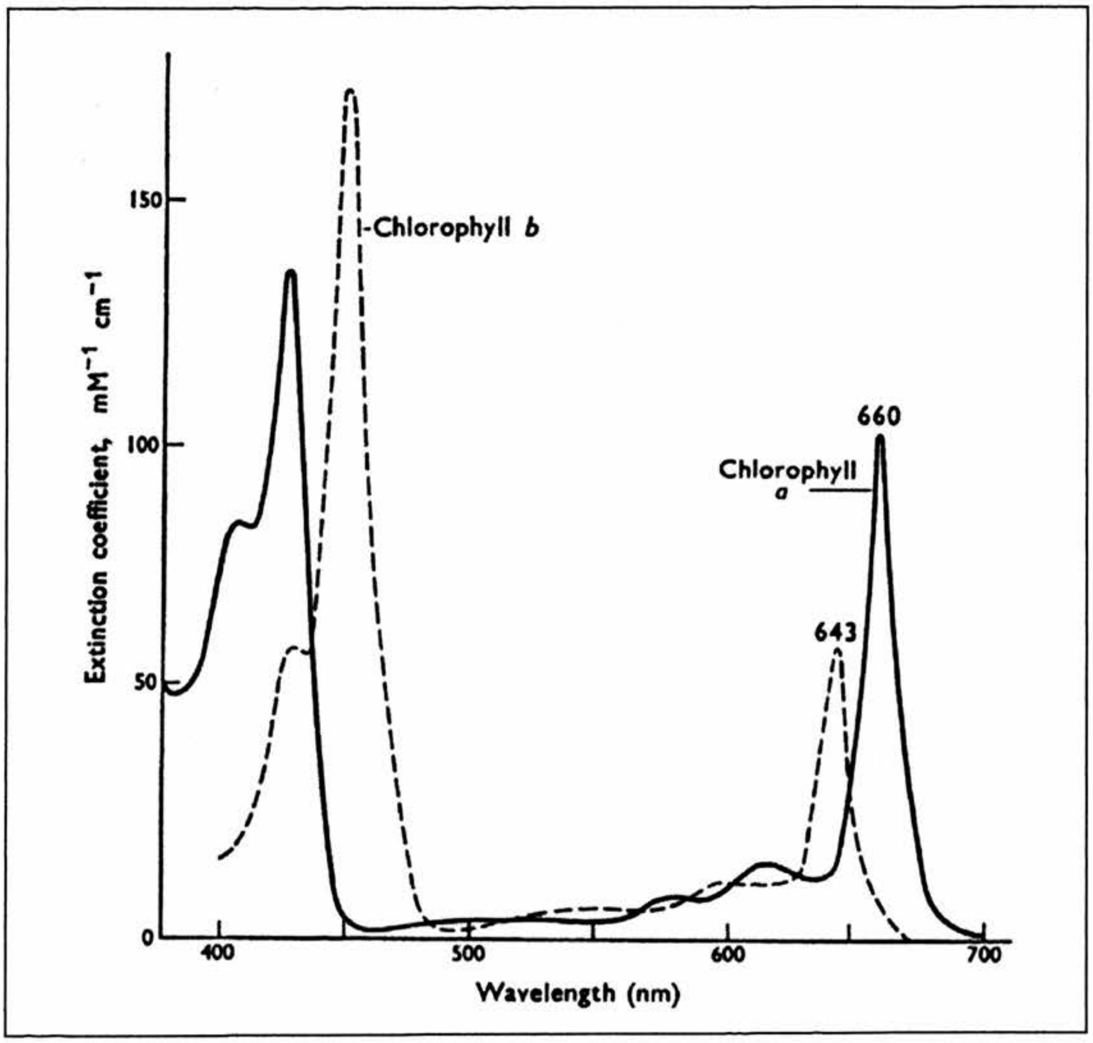


Figure 3.05. Absorbance spectra of Pigments. From Hall and Rao (1977).

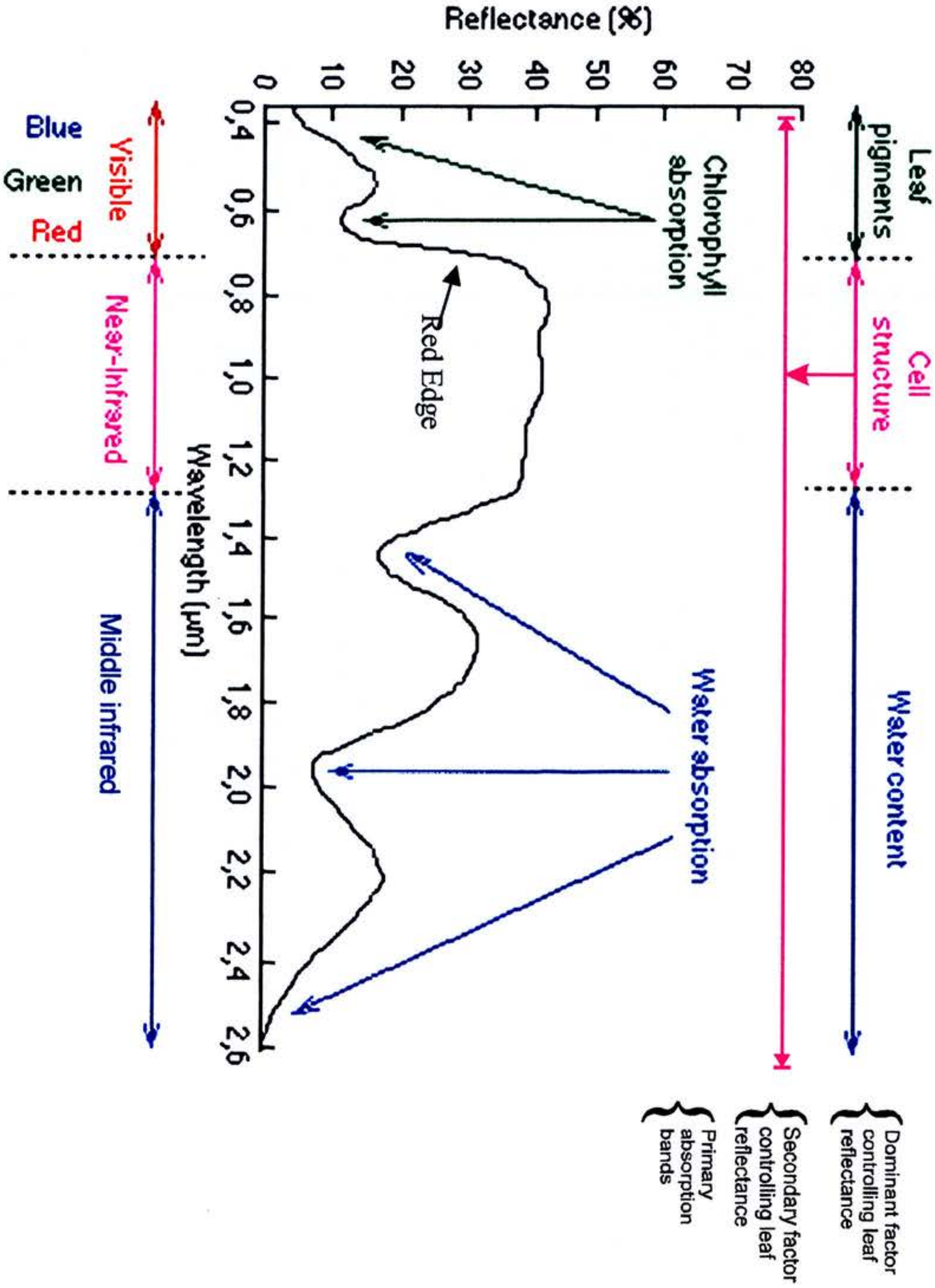


Figure 3.06 Typical reflectance spectrum of a leaf with principal causes of absorption. After Hoffer (1978). Downloaded and modified from Samson (no date).

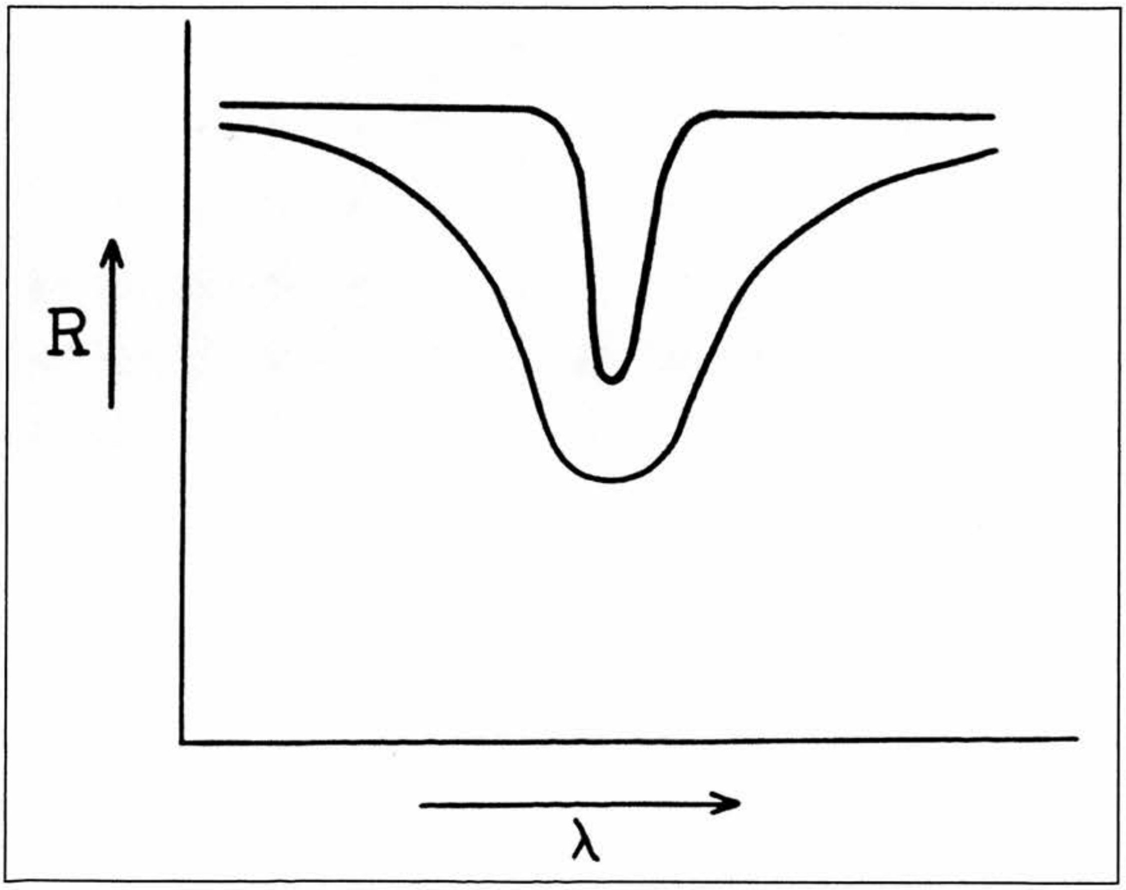


Figure 3.07. How Beers Law affects reflectance. With increased concentration or path length absorbance increases and reflectance decreases at every wavelength the molecule absorbs at. This broadens and deepens absorption features. From Salisbury *et al.*(1987)

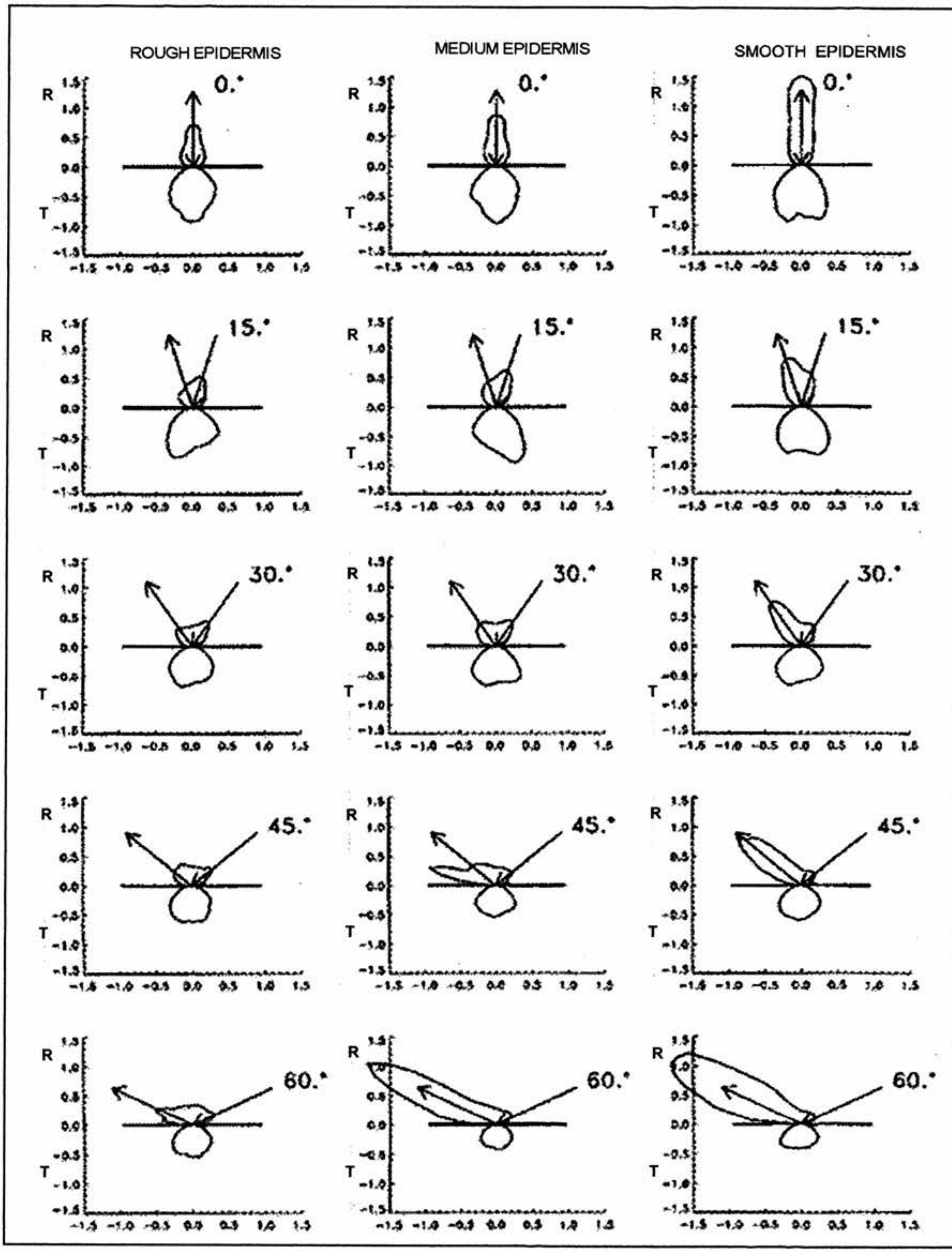


Figure 3.08. Variation in directional reflectance and transmittance with angle of incident radiation relative to different leaf surfaces. A more erect leaf such as a grass would receive incident radiation at a less perpendicular angle, (e.g. 60°). From Govaerts, (1999).

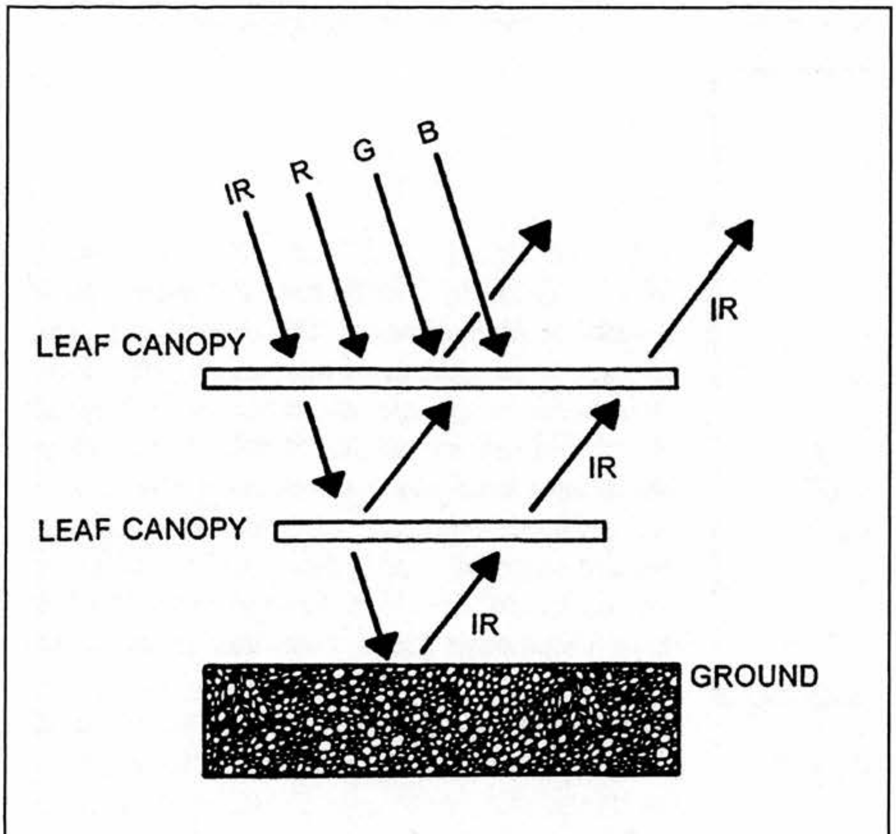


Figure 3.09. Interaction of light of different wavelengths with leaf canopies. Most red, green and blue light ("R", "G", and "B") is absorbed by photosynthetic pigments in the first layer. Near infra-red light ("IR") is not absorbed, and is transmitted or reflected from each leaf layer, and reflected from the ground and back out of the leaf canopy. From Campbell (1996)

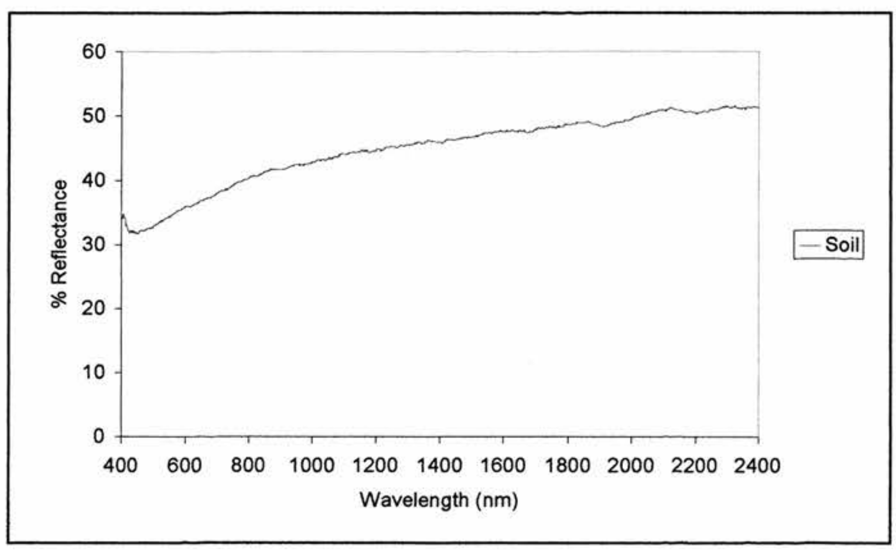


Figure 3.10. Typical soil reflectance spectrum.

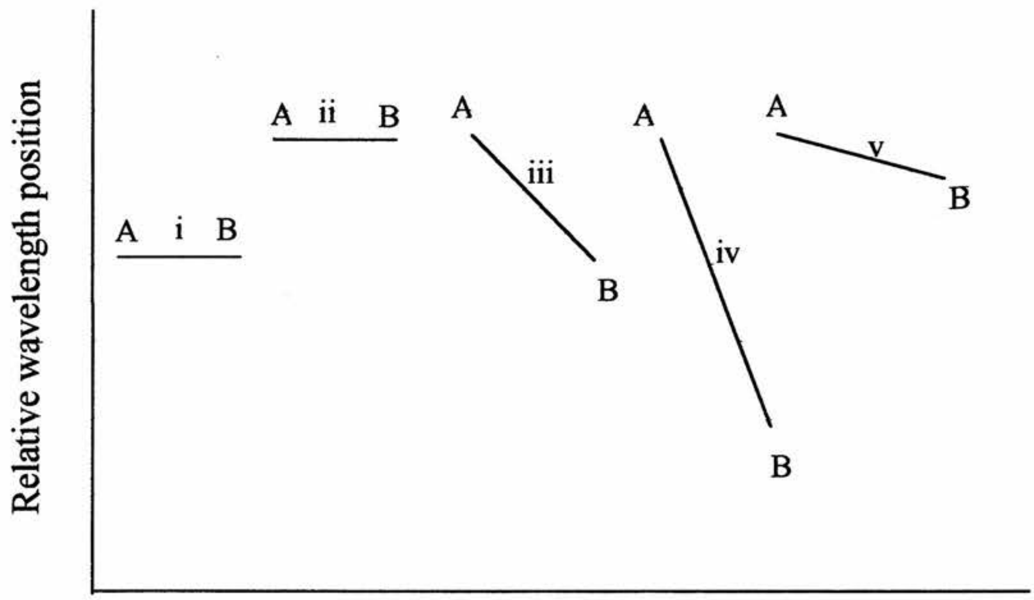
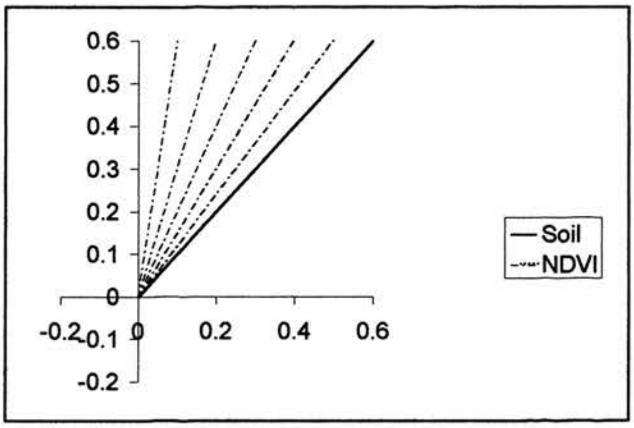
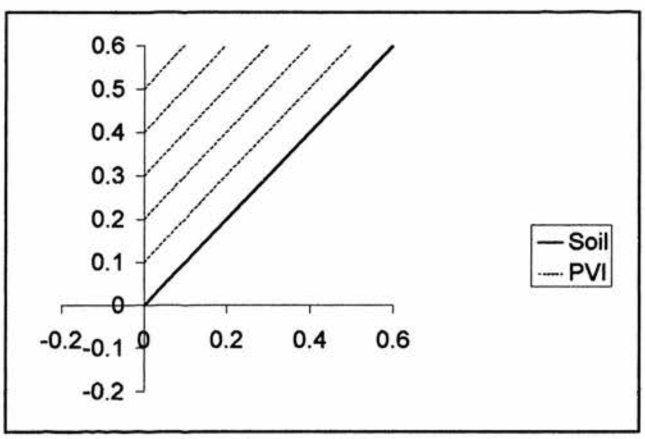


Figure 3.11. How the movement of bands used in vegetation indices affects index values. After Morain (1978) cited in Campbell (1996).

A



B



C

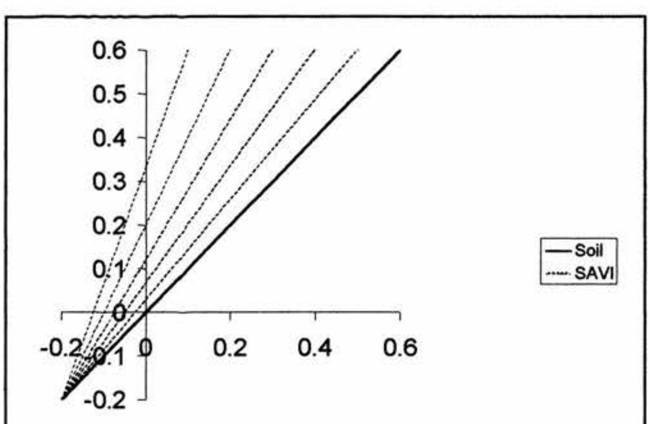


Figure 3.12. Isolines for soil and vegetation for NDVI (A); PVI (B); and SAVI (C).

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Chapter 4: Remote sensing of Leaf Reflectance

4.1 Introduction.

This chapter investigates the relationships between contamination and spectral response of leaves. Multi-species and multi-stress relationships are examined via leaf reflectance, controlling for ground, topographical and canopy architectural effects. The reflectance spectrum response of two ecotypes from two species (*Festuca rubra* and *Agrostis capillaris*), in response to three different acute stresses (zinc, copper and salt (NaCl)) was investigated. For each species one ecotype is tolerant to zinc and copper, and one is not tolerant. None of these ecotypes are known to be salt tolerant, and its inclusion here is as a general agent of stress on the plants (Taiz and Zeiger, 1991).

The plant spectral response was investigated as reflectance, vegetation indices and red edge position (REP). The effects are related to treatment and chlorophyll concentration measured in the leaves. This research had an experimental design consisting of pots of ecotypes grown in standard conditions with control and stressor treatments.

The following hypotheses were investigated:

Hypothesis 1: *The spectral response of non-tolerant grass on clean soil differs from the same grass on contaminated soil.*

Other studies have investigated this effect, although none have specifically mentioned tolerance or non-tolerance. This is because the presence of tolerance, either tolerant species or ecotypes, has been ignored by the remote sensing community. Studies have found a spectral response to stress, a change in reflectance (Carter, 1993) which can be measured as VI's (Carter and Miller, 1994) or movement of the REP (Jago *et al.*, 1999).

Hypothesis 2: *The spectral response of tolerant grass does not alter with levels of contamination that affect the spectral response of non-tolerant grass.*

This is what separates this investigation from those carried out before. Given that tolerant plants exist, and are likely to inhabit contaminated areas, is the remote sensing of contamination via location of vegetation stress a viable technique? Knowledge of the biology of tolerant plants suggests that they will not to show a physiological response to a stress which would affect less tolerant plants, so a spectral response will not be shown. Very high levels of metal may cause a stress even in tolerant plants, and such levels are investigated here.

Two assumptions are also made:

Assumption 1: *The cultivars differ only in their response to metal.*

Knowledge of the biology of ecotypes suggests that there will be some biological difference between ecotypes other than their tolerance to metal. However, this is likely to be slight and the experimental design should control for it.

Assumption 2: *Stress is caused by the contaminant.*

This is assured by the experimental design, with standardised conditions, adequate and controlled watering and feeding.

4.2 Methods

4.2.1 Choice of test species.

The main biological requirements of species for this experiment was that they must have tolerant and non tolerant ecotypes available as seed. If tolerant adult individuals were transplanted from the natural environment they may have residual tolerance from their previous location. While it would be possible to obtain tolerant adult individuals from mine sites, any seed harvested from there would be a mixture of tolerant and non-tolerant types because of gene flow (Chapter 2). It would not be possible to gather non-tolerant adults or seed from non-contaminated areas as tolerant plants, and so tolerant seeds may be present, albeit at low frequencies (McNeilly, 1968). Grown from standard seed stock there should be equal tolerance amongst each ecotype.

Suitable seed was obtained from Johnson's Seeds (London Road, Boston, Lincolnshire, PE21 8AD), *Festuca rubra* cv. Merlin (*Fr* Merlin (T)). This has been used as a tolerant ecotype in previous studies including Davies *et al.* (1991) and Powell *et al.* (1986). Johnson's seeds also provided *Festuca rubra* cv. Jupiter (*Fr* Jupiter (NT)) as a non tolerant ecotype to be used as a control. After extensive research of seed suppliers this was the only commercial source of tolerant seed. Small quantities of tolerant seed were also obtained form IGARRS in Aberystwyth by Mervynn Humphries, who supplied *Agrostis capillaris* cv. Lance (*Ac* Lance (NT)), Coginan (*Ac* Coginan (T)). *Ac* Lance (NT) was used as a non tolerant control in Cook *et al.* (1971). The ecotypes used and their respective tolerances are given in Table 4.01.

Table 4.01. Species and ecotypes used in this study with information on the metals to which they are tolerant.

Species	Ecotype (cultivar)	Metals to which the ecotype is tolerant.	Source of information.
Festuca rubra	Merlin (T)	Zn, Cu, Pb,	(Davies *et al.*, 1991), (Powell *et al.*, 1986)
	Jupiter (NT)	none	Pers. comm. Prof. A Baker, and Johnson's Seeds
Agrostis capillaris	Coginan (T)	Cu, Zn, Pb	Pers. comm. M Humphreys
	Lance (NT)	None	(Cook *et al.*, 1971), Pers. comm. M. Humphreys

NB. *Fr* Merlin (T) is no longer commercially available.

4.2.2 Experimental design.

Free draining 4" diameter plastic plant pots were filled with John Innes No. 1 potting compost and sown with a low density of seed (approx. 30 per pot). Four ecotypes were used, with six treatments for each ecotype (control, low copper, high copper, low zinc, high zinc and salt). Each treatment had two replicates. Each pot had the seeds of only one ecotype, and the pots were arranged in random order. The plants were grown in a heated, lit greenhouse. The pots were moved during the growth period so growing conditions experienced by the plants were standard. Pots were watered frequently and monitored for weeds. The growth period for this experiment was 42 days.

After 35 days the pots were contaminated as shown in Table 4.02 using a fine rose watering can. The use of a fine rose minimised immediate run through of contaminants and allowed direct foliar absorption. Contaminants applied were copper, zinc and salt. The concentrations were decided upon from various sources.

Ross (1994) lists zinc as having a toxic effect above 400 mg kg^{-1}, and copper above 125 mg kg^{-1}(dry weight of soil). The UK Department of environment listed "Trigger concentrations" (the threshold at which a site is considered contaminated) as being 130 mg kg^{-1} for copper and 300 mg kg^{-1} for zinc. Salt concentration is measured as the salinity of the soil water. Salt (NaCl) was added as a solution of 10g l^{-1} to replace the soil water.

Table 4.02. Concentrations of contaminants applied to pots to engender acute toxicity.

Contaminant	Chemical form	Soil concentration ($\mu g\ g^{-1}$ dry weight) Low	High
Copper	$CuSO_4 . 7\ H_20$	125	250
Zinc	$ZnSO_4$	300	600

4.2.3 Pigment Analysis

Photosynthetic pigment analysis was determined at the end of the growth period (day 42). A sample of secondary leaves were harvested from each pot. This ensured that all leaves were at the same stage of growth so pigment concentration was related to stress, and was not caused by differing ages of leaves. Enough leaves were harvested to ensure that two pigment estimates could be made per pot, 4 per treatment. They were stored in dark, cold conditions and transported to the laboratory. The pigment analysis followed the methods of (Sestak *et al.*, 1971). The leaves were weighed, then ground in a mortar and pestle with a pinch of washed sand and $MgCO_3$ with approx. 5 ml 80% acetone. When there were no more visible pieces of grass, the contents of the mortar were washed into a graduated centrifuge tube. The mortar and pestle were then rinsed with more 80% acetone so that the volume in the tube was no more than 10 ml, and there was no more residue in the mortar or on the pestle. The tubes were then centrifuged at 3000 rpm for 5 minutes. The supernatant liquid was transferred to a 1 cm cuvette and analysed in a spectrophotometer (Perkin-Elmer

Lamda 40 UV/Vis). A full scan of transmittance from 400-1100 nm was undertaken. The concentration of chlorophylls a and b were separately calculated using equations from (Lichtenthaler, 1987), as mg g^{-1} fresh weight of the leaf. Leaf samples were also dried to ascertain a fresh:dry weight ratio, and this was used to calculate chlorophyll concentration as mg g^{-1} dry weight.

4.2.4 Leaf spectral analysis.

Leaf spectral data were measured using a Labsphere RSA PE20 50mm integrating sphere attached to a UV/Vis/NIR Perkin-Elmer Lamda-40 spectrophotometer. All leaves measured were secondary leaves, thus ensuring that they were equally developed, as maturity can greatly effect remote sensing results (Gausman and Quisenberry, 1990). The leaf spectral data were interpolated to 1nm intervals using a computer program written by Tim Malthus, Edinburgh University. The ports of the integrating sphere are 11mm diameter, which creates problems when measuring the reflectance of leaves whose dimensions are smaller than the size of the port (e.g. grass blades or needles). If the leaves are arranged over the sample port so they overlap the reflectance measured is a mixture of single and multiple leaf layers, which was unacceptable. If the leaves are arranged over the port so they do not overlap, the reflectance is an unknown combination of leaf and gap. However, using the latter method, and by a measuring of the size of the light beam and the proportion of leaf and gap within it, an estimate can be made of the reflectance value that would have been measured had the leaf covered the whole light beam (Mesearch *et al.*, 1999).

The measurement of the size of the light beam was carried out following the method devised by (Shaw, 2001). Slide sized card was coated with Liquid Light, a silver-halide photographic emulsion. This slide was mounted in the integrating sphere with the spectrophotometer set to white light for 5 seconds. The card was then processed to fix the image to it, and the image of the light beam was cut out to form a mask, the use of which is described below.

Using conifer needles, (Mesearch *et al.*, 1999) developed the method used here for relating the mixture of spaces and leaves to the equivalent of a whole scene comprised solely of leaf. The integrating sphere has a slide holder, so holders were made to these dimensions where the position of the grass blades relative to the light beam remained constant. The blades were mounted on slide sized card, and the reflectance measured using the integrating sphere. The light beam mask was then laid over the card of mounted blades, and a digital image made using an Epson flat bed scanner (at 600 dpi) so the area of needles and gap exposed to the light beam was known. These scanned images were imported into the ER Mapper image processing software and used to estimate the proportion of gap and leaf (gap fraction), which was subsequently used to estimate the reflectance if the gaps were not present using Equation 4.01.

Equation 4.01 from (Mesearch *et al.*, 1999).

$$\rho = \frac{\rho_{total}}{(1 - GF)}$$

where

ρ = reflectance of grass leaves

$\rho_{\text{total}} = R_{\text{total}} - \text{STR} / \text{REF} - \text{STR}$

R_{total} = reflected radiation from the sample

STR = stray light radiation

REF = reference radiation

GF = gap fraction

The stray light term was ignored as the integrating sphere was in a light trap.

4.2.5 Statistical analysis

The spectral reflectance data were imported into Microsoft Excel which was used for all statistical analysis. As Excel contains only a limited number of statistical tests which have unwieldy data formatting requirements, spreadsheets were designed with user entered statistical formulae from Zar (1984).

Samples of the data were taken to examine whether the assumptions of parametric statistical tests were met. Most samples did not have a normal distribution or equal variances. It was not feasible to test all the data for conformation to the requirements of parametric tests, and it would not be suitable to use parametric tests on some parts, and not on others, so non-parametric statistical tests were used for all analysis. Non-parametric tests are nearly as powerful as parametric tests (e.g. the Kruskal-Wallis test is 95% as powerful as ANOVA) and, if parametric tests are inappropriate as in this case, they will be more powerful (Zar, 1984). Non-parametric tests are rare in remote sensing statistical analysis, however, but perhaps their use should be more widespread.

For two-sample testing the Mann-Whitney U test was used, which is a non-parametric analogue of the two-sample *t* test (Zar, 1984). For multiple sample testing the Kruskal-Wallis test was used, which is a non-parametric analogue of the ANOVA test (Zar, 1984). If the null hypothesis was rejected by the Kruskal-Wallis test a non-parametric multiple comparison test was used to investigate between which samples the significant differences occurred. The comparison test used was that proposed by Zar (1984, pp199 - 201), an unnamed technique similar to the Tukey test. The significance level used in all tests was $\alpha=0.05$.

4.2.6 Data Analysis

The reflectance data were first examined by statistically testing the differences between treatments at each wavelength. This analysis can show regions of the spectrum where reflectance is responding to treatment. Statistical results are shown on the same figure. Data are presented as reflectance differences (after Carter and Miller, 1994) which is the difference between treatments in percentage reflectance units at each wavelength. This is essentially a technique to improve contrast.

Vegetation indices were also used, both obtained from the literature and developed as part of this study. Indices were chosen for their applicability to stress detection, most having been developed specifically for that purpose (Table 4.03). The results of the indices were analysed using the statistical techniques described earlier for differences between treatment reflectances.

The first derivative of reflectance was also calculated, using a program provided by Tim Malthus, Edinburgh University. This used least squares polynomial smoothing (Savitzky and Golay, 1964). After qualitative analysis, a smoothing width of 9 nm was used. A smaller smoothing width resulted in derivative curves with multiple peaks, and a larger smoothing width gave a "rounded" appearance to the peaks. The red-edge position is defined here as the peak of the first derivative curve ca. 700nm. The derivative results were analysed using the previously described statistical techniques.

Table 4.03. Vegetation stress indices used in this study.

Author	Name of Index	Formulae	Application
(Blackburn, 1998b)	PSSRa	800/675	chlorophyll a
	PSSRb	800/650	chlorophyll b
	reformed PSSRa	800/680	chlorophyll a
	reformed PSSRb	800/635	chlorophyll b
	PSSRc	800/500	carotenoid
	PSNDa	$\frac{800-680}{800+680}$	chlorophyll a
	PSNDb	$\frac{800-635}{800+635}$	chlorophyll b
(Carter and Miller, 1994)		695/760	
Malthus et al. 1995		425/470	stress
		446/477	stress
		541/836	stress
		818/538	stress
		818/713	stress
(Penuelas *et al.*, 1994)	WBI	970/900	
	PRI	$\frac{550-530}{550+530}$	
	NPCI	$\frac{680-430}{680+430}$	
This Study	GP/RT	GP/RT	stress

PSSR = Pigment Specific Simple Ratio; PSND = Pigment Specific Normalised Difference; a = Chlorophyll a; b = Chlorophyll b; c = Carotnoids; WBI = Water Based Index; PRI = Physiological Reflectance Index; NPCI = Normalise Pigments Chlorophyll ratio Index

4.3 Results and Discussion

It was shown in Chapter 1 that for remote sensing to provide a valid methodology for detection of stress a linear or curvilinear relationship should be proven between the amount of stress a plant experiences and its reflectance, or component of its reflectance. The direction of the response has to remain constant to allow a quantification of the amount of stress to be made, and to lessen the possibility of false negative results happening.

What was also being tested here was whether the response will vary in different ecotypes. A limit of the usefulness of remote sensing in the natural environment is a need for community information and characterisation of the reflectance properties of those species (Clark *et al.*, 1995). If ecotype information is needed in addition remote sensing's usefulness becomes more limited, as ecotype identification and characterisation in natural ecosystems would be an extremely difficult task.

4.3.1 Prediction of results

From the biological understanding of tolerance, its influence on the ability of plants to mitigate plant stress, and the hypotheses stated earlier, a number of predictions of expected results can be made, and tested for (Table 4.04). The predictions for the non-tolerant ecotypes were the same as each other, as were those of the tolerant ecotypes. The predictions for the non-tolerant ecotypes (*Fr* Jupiter (NT) and *Ac* Lance (NT)) were that low levels of metals should have an effect on the plant spectral properties, with high levels having a greater effect. The tolerant ecotypes (*Fr* Merlin (T) and *Ac* Coginan(T)) were predicted to have no spectral response to contamination at low levels of metal, and possibly having a response at high levels. This response was harder to predict as even at high metal concentrations the tolerance may negate any effect of the metal. All ecotypes were predicted to have a stress response to salt, as none were reported as being tolerant to it.

Table 4.04. Predicted responses based on knowledge of tolerance for all ecotypes. "δ" indicates a general movement relative to the control but does not predict the direction of movement. "δδ" represents a greater response. The same number of "δ" represents a similar level of response between treatments. "-" indicates no difference to the control. "δ /-" means either could happen.

	Predicted treatment responses relative to the control				
Prediction for:	Lo Cu	Hi Cu	Lo Zn	Hi Zn	Salt
Fr Jupiter (NT)	δ	δδ	δ	δδ	δ
Fr Merlin (T)	-	δ/-	-	δ/-	δ
Ac Lance (NT)	δ	δ	δ	δ	δ
Ac Coginan (T)	-	δ/-	-	δ/-	δ

4.3.2 Chlorophyll results

The results of the chlorophyll analysis were not statistically testable due to a lack of sample leaves available in some treatments. The leaves for chlorophyll analysis were taken after the leaf spectra were recorded, which took precedence for statistically testable results (i.e. for replicates). As such, from some highly stressed treatments only 1 or 2 batches of secondary leaves were left for pigment analysis. The results did show general patterns however, and were useful in giving an indication of the effects of the treatments on the chlorophyll *a* and *b* concentrations. Unless otherwise stated both chlorophyll *a* and *b* are referred to simply as chlorophyll for the rest of this chapter because of the similarity of their responses. They were compared with the predictions based on biology, and were incorporated into the same tables to predict reflectance response.

Figure 4.01 shows the change in chlorophyll concentration with treatment for *Fr* Merlin (T). The low copper, low zinc and salt treatments did not affect chlorophyll concentration, while the high copper and high zinc treatments had a lower chlorophyll concentration than the control. As expected (Table 4.05) there was no response of chlorophyll concentration to either low metal treatment. There were responses to the high metal treatments, indicating that the level of metal concentration was high enough to cause stress effects in this tolerant ecotype. The salt treatment did not seem to affect chlorophyll concentration, however, indicating either a level of tolerance in this ecotype to salt, or the salt concentration being too low to elicit an effect.

Table 4.05. Predicted reflectance results based on knowledge of tolerance and chlorophyll results for *Fr* Merlin (T). "δ" indicates a movement relative to the control. "δδ" represents a greater response. The same number of "δ" represents a similar response between treatments. "-" indicates no difference to the control. "δ/-" means either could happen.

	Predicted treatment reflectance responses relative to the control				
	Lo Cu	Hi Cu	Lo Zn	Hi Zn	Salt
Prediction from Biology of tolerance	-	δ/-	-	δ/-	δ
Chlorophyll results	-	δ	-	δ	-

For *Fr* Jupiter (NT) all the metal treatments reduced the chlorophyll concentration to similar levels relative to the control, but the effect of the salt treatment was uncertain (Figure 4.02). The expected decrease in chlorophyll concentration with high treatments relative to low metal treatments was not apparent (Table 4.06). The salt treatment did not have an effect on chlorophyll concentration, unlike the prediction based on tolerance (Table 4.06). This indicated that the treatment either stressed plants in a way that did not alter pigment composition, or that *Fr* Jupiter (NT) was tolerant to it.

Table 4.06. Predicted reflectance results based on knowledge of tolerance and chlorophyll results for *Fr* Jupiter (NT). "δ" indicates a movement relative to the control. "δδ" represents a greater response. The same number of "δ" represents a similar response between treatments. "-" indicates no difference to the control. "δ/-" means either could happen.

	The treatments reflectance were predicted to respond as following relative to the control				
Prediction from:	Lo Cu	Hi Cu	Lo Zn	Hi Zn	Salt
Biology of tolerance	δ	δδ	δ	δδ	δ
Chlorophyll results	δ	δ	δ	δ	-

Comparing the two ecotypes of *Festuca rubra* the control chlorophyll concentrations were similar (approx. 15 mg g^{-1}). The high metal treatments lowered the chlorophyll concentrations to similar values in both ecotypes (approx. 3 mg g^{-1}). The low metal treatments resulted in the same chlorophyll concentrations as the high treatments in *Fr* Jupiter (NT), but were the same as the control in *Fr* Merlin (T). Salt appeared to have no effect on chlorophyll concentration in either ecotype, indicating either too low a treatment, tolerance to it in both or salt stress affecting parts of the plant other than chlorophyll concentration.

The chlorophyll results for Ac Coginan (T) are shown in Figure 4.03. As expected (Table 4.07) there was little response to low copper, while the high copper and high zinc treatments chlorophyll concentration did decrease. The low zinc treatment showed a marked decrease in chlorophyll concentration, while the salt treatment showed no response, neither of which were expected (Table 4.07). The lack of a salt response can be explained as for the *Fr* ecotypes, while the decrease in chlorophyll content with low zinc can only be attributed to *Ac* Coginan (T) not being tolerant to zinc at the concentrations levels used.

Table 4.07. Predicted reflectance results based on knowledge of tolerance and chlorophyll results for *Ac* Coginan (T). "δ" indicates a movement relative to the control. "δδ" represents a greater response. The same number of "δ" represents a similar response between treatments. "-" indicates no difference to the control. "δ/-" means either could happen.

	The treatments reflectance were predicted to respond as following relative to the control				
Prediction from:	Lo Cu	Hi Cu	Lo Zn	Hi Zn	Salt
Biology of tolerance	-	δ/-	-	δ/-	δ
Chlorophyll results	-	δ	δ	δ	-

Ac Lance's (NT) chlorophyll results are given in Figure 4.04. This non-tolerant ecotype showed chlorophyll concentrations reduced to similar levels in all metal treatments relative to the control. The effect of salt on chlorophyll content was indeterminable as the range was too high. Apart from the salt treatment all had the expected effect on chlorophyll concentration, although the level of effect did not change with higher metal applications (Table 4.08).

Table 4.08. Predicted reflectance results based on knowledge of tolerance and chlorophyll results for *Ac* Lance (NT). "δ" indicates a movement relative to the control. "δδ" represents a greater response. The same number of "δ" represents a similar response between treatments. "-" indicates no difference to the control. "δ/-" means either could happen. "?" means unknown.

	The treatments reflectance were predicted to respond as following relative to the control				
Prediction from:	Lo Cu	Hi Cu	Lo Zn	Hi Zn	Salt
Biology of tolerance	δ	δδ	δ	δδ	δ
Chlorophyll results	δ	δ	δ	δ	?

The two *Agrostis capillaris* ecotypes had similar control concentrations (15 mg g^{-1}). *Ac* Coginan (T) high copper and both zinc treatments, and all metal treatments for *Ac* Lance (NT) were lowered to similar levels (2 to 3 mg g^{-1}). *Ac* Coginan's (T) low copper chlorophyll concentration was between that of the control and high copper, and it had no response on chlorophyll from salt.

Comparing all chlorophyll concentration on the same graph gives us an indication of likely reflectance responses assuming that reflectance properties were related to chlorophyll concentration (Figure 4.05, Table 4.09). These suggested that the control reflectance for all ecotypes should be similar. The low copper treatment should give a different reflectance response between the non-tolerant and tolerant ecotypes with the tolerant ecotypes being similar to the control. The high copper treatment should have the same reflectance response in all ecotypes. The low zinc treatment should have a different reflectance response in *Fr* Merlin (T) (similar to the control) relative to the other ecotypes, and all ecotypes reflectance should be similar in the high zinc treatment. The salt response was less clear, but would be expected not to be too dissimilar to the control reflectance for all ecotypes.

Table 4.09. Predicted visible reflectance results based on chlorophyll results for all ecotypes. "δ" indicates a movement relative to the control. "δδ" represents a greater response. The same number of "δ" represents a similar response between treatments. "-" indicates no difference to the control. "δ/-" means either could happen. "?" means unknown.

	The treatments reflectance were predicted to respond as following relative to the control				
	Lo Cu	Hi Cu	Lo Zn	Hi Zn	Salt
Fr Jupiter (NT)	δ	δ	δ	δ	-
Fr Merlin (T)	-	δ	-	δ	-
Ac Lance (NT)	δ	δ	δ	δ	?
Ac Coginan (T)	-	δ	δ	δ	-

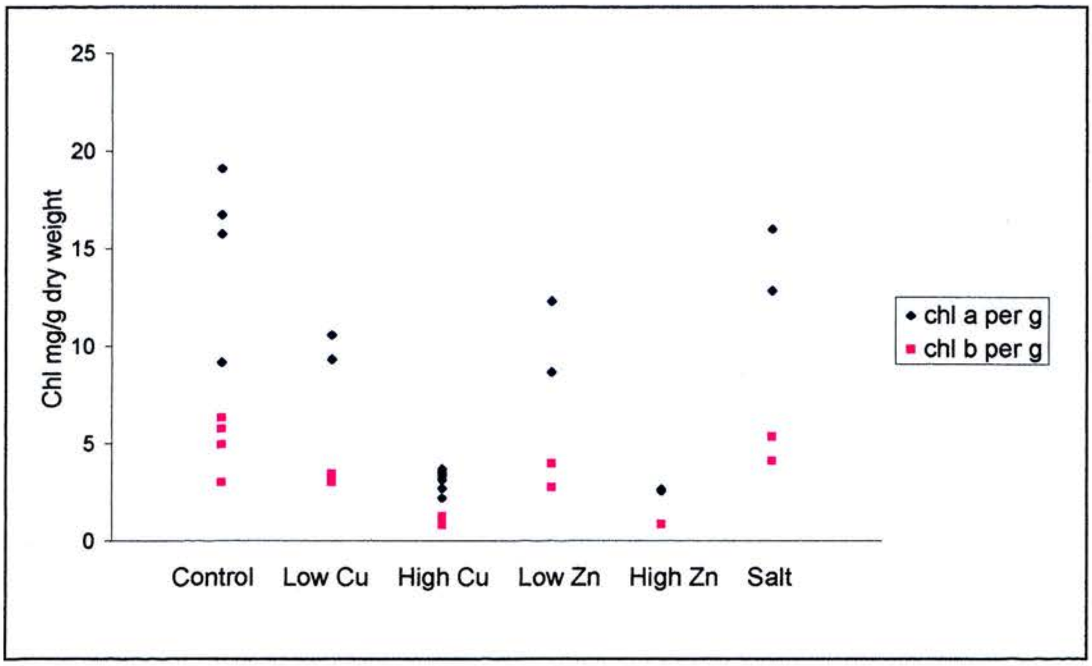

Figure 4.01. *Festuca rubra* Merlin (T) chlorophyll concentration results.

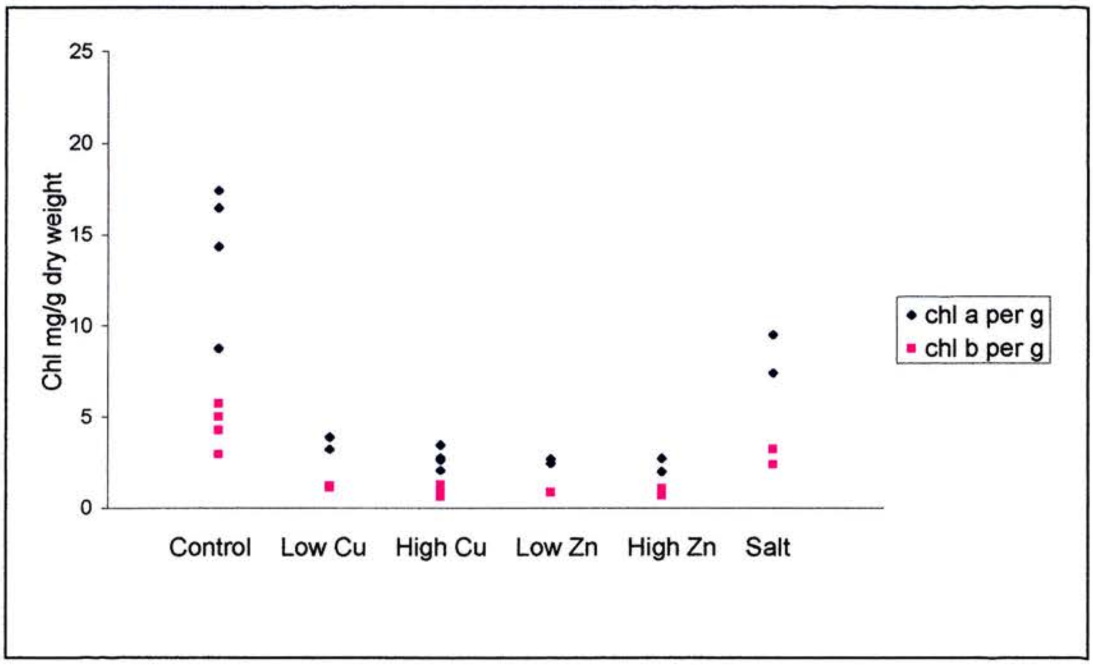

Figure 4.02. *Fr* Jupiter (NT) chlorophyll concentration results.

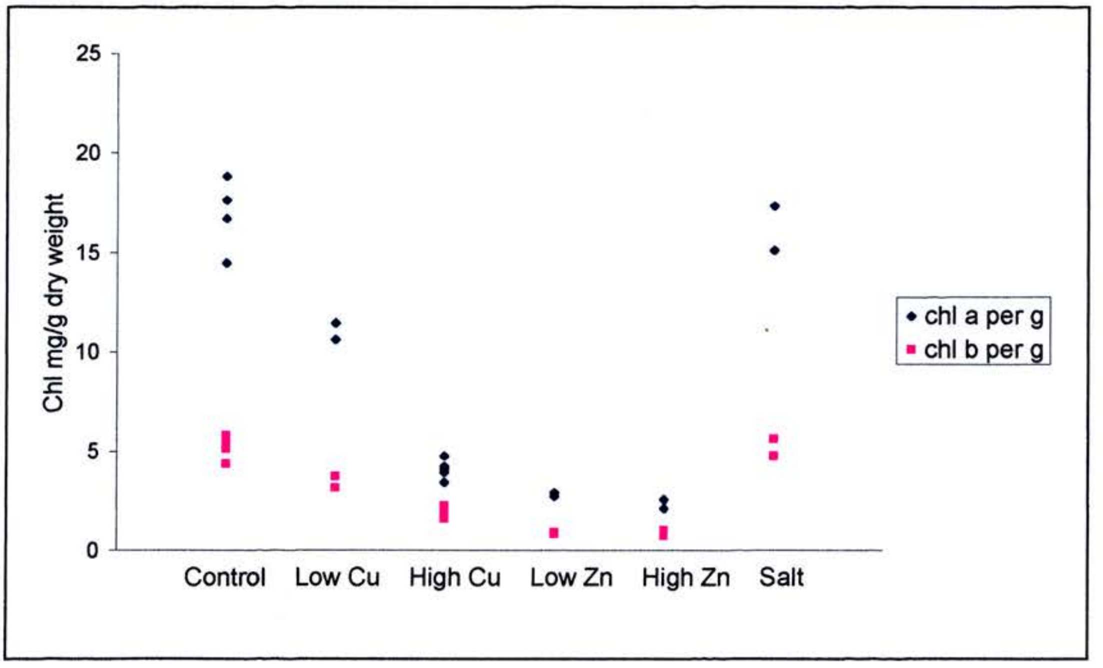

Figure 4.03. *Ac* Coginan (T) chlorophyll concentration results

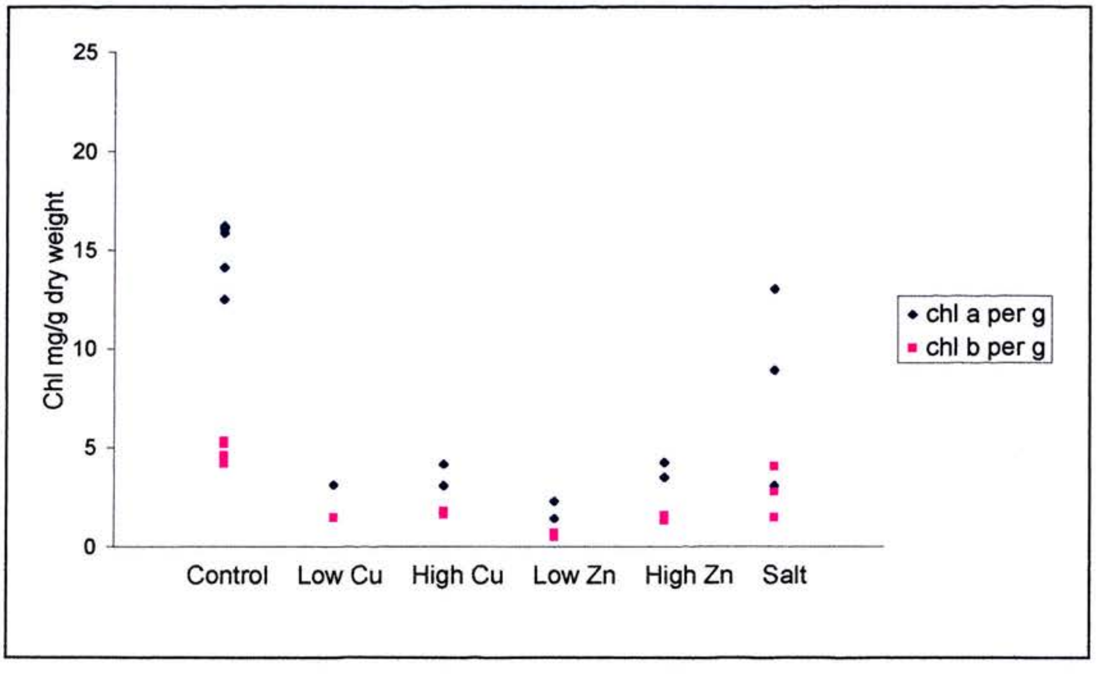

Figure 4.04. *Ac* Lance (NT) chlorophyll concentration results

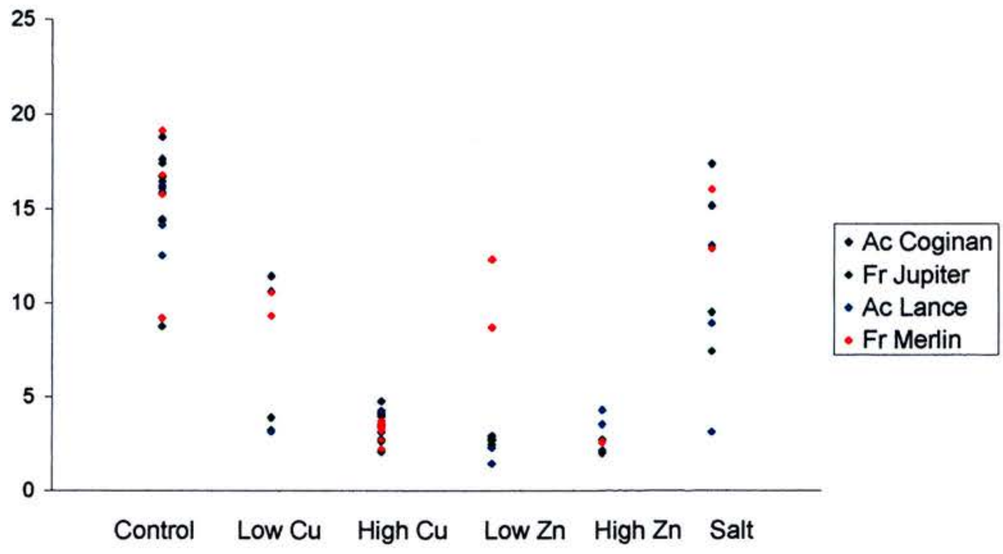

Figure 4.05. Chlorophyll *a* concentrations for all ecotypes and treatments.

4.3.3 Reflectance results

The reflectance analysis was carried out in order to assess the influence of treatment on the spectral properties of the plants, and to discover patterns and wavelengths that may prove useful if integrated into indices. The average of all replicates for each treatment was used in all figures. The reflectance data are presented as reflectance differences in Figures 4.06 to 4.09 (Carter, 1993). This represented the difference between the control and the metal treatments. As such the control can be taken as zero, and at any wavelength a reading of -1 shows that treatment had a percentage reflectance 1% lower than the control. This technique enabled small differences in reflectance between treatments to be made more obvious. The results of statistical tests are presented on the same graphs, using the same colour codes. The presence of a line at the bottom of the graph means that at that wavelength there was a significant difference ($p \leq 0.05$) between the control and that treatment. The results are reported with the observation that while non-significant differences were statistically the same as the control, the presence of a pattern or direction of movement may be useful in the formulation of an index. As such some non-significant results are discussed.

As Chapters 1 and 3 introduced, remote sensing requires that a response to the same stress should be similar in all individuals, ecotypes and species. The ideal response should be linear or curvilinear, so that a higher dosage of metal changes the spectra proportional to a lower dosage. The response to the same stress in different individuals, ecotypes, or species should ideally also be the same, although the response to different stresses may alter.

These results were tested against the predictions made in previous sections based on knowledge of the biology of tolerance, and the chlorophyll results. The biology predictions were tested against the whole spectra, while the chlorophyll predictions were tested only against the visible parts of the spectrum, as this is where chlorophyll concentration affects reflectance.

Figure 4.06 shows the reflective difference results for the ecotype *Fr* Jupiter (NT). Across the visible region of the spectrum there was a flat response, meaning that while the treatments reflectance may have differed from the control, the response remained constant. Into the near infrared, there was a region of transition ca. 700nm and a similarly flat response beyond this wavelength. Both copper treatments were significantly different from the control in the visible region, while in the near-infrared (NIR) only the low copper treatment was different from the control. The high zinc treatment showed a small region of significant difference while the low zinc treatment was significantly different from the control over much of the visible region. The salt treatment was significantly different from the control across the visible and in some regions of the NIR. The spectral response of the salt treated plants was different to the metal treated plants in the visible region, exhibiting lower reflectance than the control, while in the NIR only it and the high copper treatment were lower than the control. This decrease in reflection could be due to an increase in refraction, chlorophyll results suggest that a change in chlorophyll concentration is not the cause. Carter (1993) found an increase in reflectance with higher salinity, highlighting the diverse responses of different species to the same stressor.

The reflectance results showed no response similar to that expected from the predictions based on tolerance at any wavelengths (Table 4.10) i.e. both high treatments were predicted to respond more than the low treatment for both metals, this did not happen. There was a small wavelength section between 533-550nm where the copper treatments alone responded like this, although there was no significant difference between treatments. The prediction based on chlorophyll results was that all metal treatments should of had similar reflectance in the visible region of the spectrum, and salt should be similar to the control (Table 4.10). There were regions where all four metal treatments had a similar reflectance, from 450-500nm, and 670-690 nm (the absorption maxima of chlorophyll), but here the salt treatment was very different to the control.

Table 4.10. Predicted reflectance results based on knowledge of tolerance and chlorophyll results for *Fr* Jupiter (NT). "δ" indicates a movement relative to the control. "δδ" represents a greater response. The same number of "δ" represents a similar response between treatments. "-" indicates no difference to the control. "δ/-" means either could happen.

Prediction from:	The treatments reflectance were predicted to respond as following relative to the control Lo Cu	Hi Cu	Lo Zn	Hi Zn	Salt
Biology of tolerance	δ	δδ	δ	δδ	δ
Chlorophyll results	δ	δ	δ	δ	-

For *Fr* Merlin (T) less wavelengths showed a significant difference between the treatment and control reflectance than for the *Fr* Jupiter (NT) treatments (Figure 4.07). Different treatments also showed a more similar pattern to each other. The general pattern of reflectance difference was of a reduction in the green region, an increase relative to that in the red, and decreased into the NIR. In a standard reflectance graph this is seen as a flatter response than the control in the visible region. While the low zinc treatment showed a significant response in the visible and NIR, the high zinc showed no significant differences. The copper treatments responded in a similar manner to each other in the visible part of the spectrum, while in the NIR their responses were very different.

While the low zinc reflectance was significantly different from the control across many wavebands, the high treatment was not, which was not as either the biology or chlorophyll analysis would have predicted (Table 4.11). The copper treatments did seem to respond as predicted in the 550-570 and 620-700nm regions.

Table 4.11. Predicted reflectance results based on knowledge of tolerance and chlorophyll results for *Fr* Merlin (T). "δ" indicates a movement relative to the control. "δδ" represents a greater response. The same number of "δ" represents a similar response between treatments. "-" indicates no difference to the control. "δ/-" means either could happen.

	The treatments reflectance were predicted to respond as following relative to the control				
Prediction from:	Lo Cu	Hi Cu	Lo Zn	Hi Zn	Salt
Biology of tolerance	-	δ	-	δ	δ
Chlorophyll results	-	δ	-	δ	-

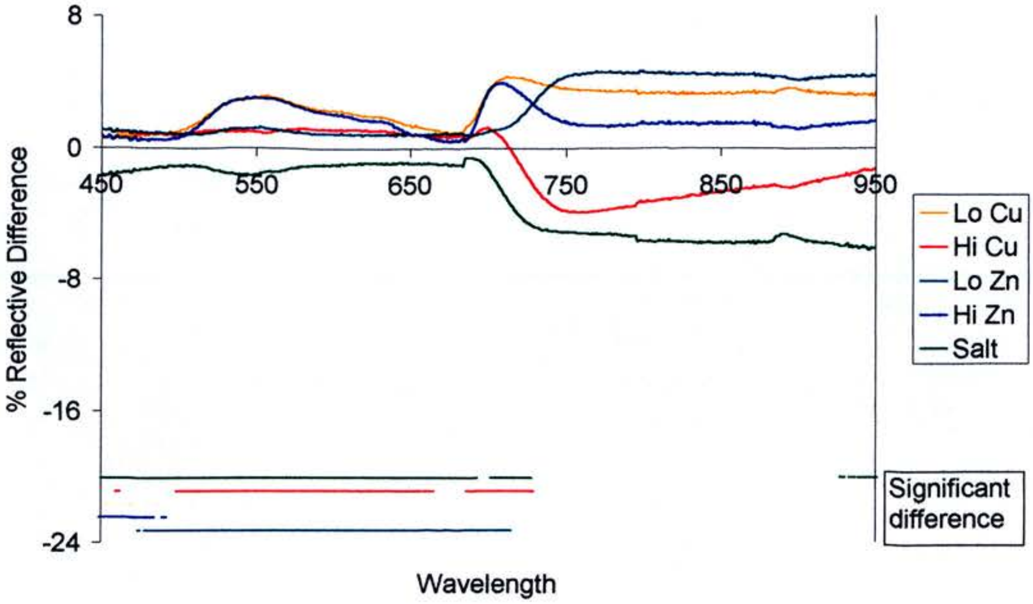

Figure 4.06. Reflective difference of *Fr* Jupiter (NT) treatments relative to the control. Lower lines (same colour coding) show significant Mann-Whitney U results between the control and that treatment.

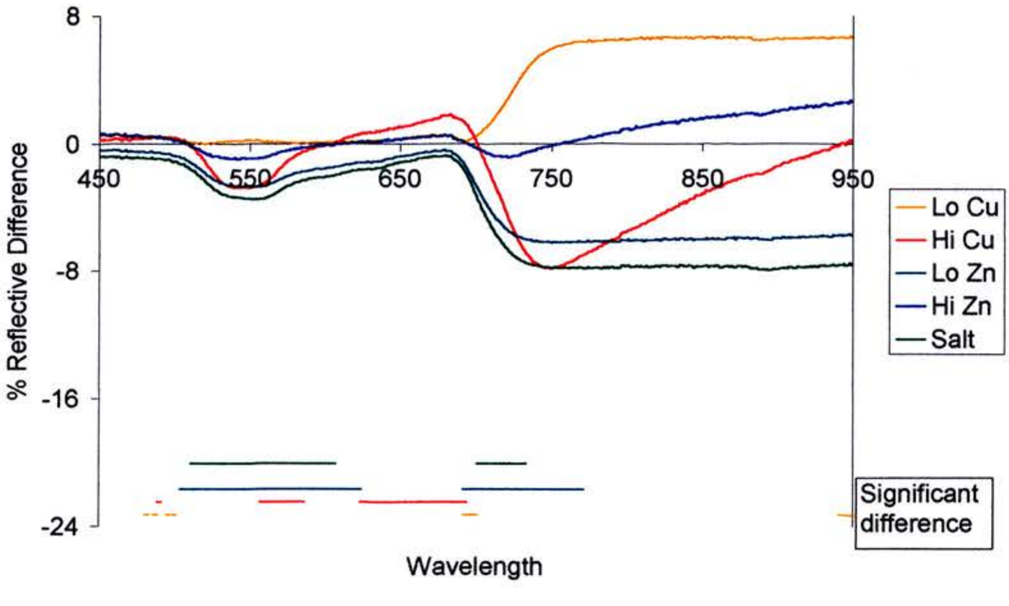

Figure 4.07. Reflective difference of *Fr* cv. Merlin (T) treatments relative to the control. Lower lines (same colour coding) show significant Mann-Whitney U results between the control and that treatment.

The reflectance difference results for *Ac* Coginan (T) showed a broadly similar pattern to each other (Figure 4.08). There was generally a decrease in reflectance in the green region relative to the control, and reflectance was more similar to the control in the red. In the NIR reflectance was markedly reduced relative to the control in all metal treatments apart from low copper. The salt treatment reflectance was significantly less than the control across all wavelengths, which while predicted from the tolerance (Table 4.12), was not predicted to happen based on the chlorophyll results. The low zinc treatment showed a significant difference (lower than the control), across most wavelengths, while the high copper treatment was significantly lower than the control only in the NIR. The order of the pattern was not as would be expected (Table 4.12). The high treatments were not more different from the control than the low treatments, apart from copper in the near infra-red.

Table 4.12. Predicted reflectance results based on knowledge of tolerance and chlorophyll results for *Ac* Coginan (T) "δ" indicates a movement relative to the control. "δδ" represents a greater response. The same number of "δ" represents a similar response between treatments. "-" indicates no difference to the control. "δ/-" means either could happen.

	The treatments reflectance were predicted to respond as following relative to the control				
Prediction from:	Lo Cu	Hi Cu	Lo Zn	Hi Zn	Salt
Biology of tolerance	-	δ	-	δ	δ
Chlorophyll results	-	δ	δ	δ	-

For *Ac* Lance (NT) the salt treatment exhibited a significantly lower reflectance than the control across most of the spectrum measured (Figure 4.09). The high copper treatment had the largest variation in reflective difference across the spectra, being lower in the green and significantly higher in the red (i.e. the actual reflectance in the visible was flatter than the control), and it was also very different from the control in the NIR. The low copper treatment showed much less difference from the control and a different pattern to the high copper. The zinc treatments across most of the spectrum showed an increase or no change in reflectance difference with increasing metal application.

Results predicted from biology for *Ac* Lance (T), a greater response to high stress than low (Table 4.13), were found from 525-568nm, and at most wavelengths from 710-950 nm. Salt was different from the control across nearly the whole spectrum, which was different from the prediction based on chlorophyll results that there should be no difference in reflectance across the visible spectrum. The metal treatments were not at similar reflectances to those predicted at any wavelengths in the visible.

Table 4.13. Predicted reflectance results based on knowledge of tolerance and chlorophyll results for *Ac* Lance (T). "δ" indicates a movement relative to the control. "δδ" represents a greater response. The same number of "δ" represents a similar response between treatments. "-" indicates no difference to the control. "δ/-" means either could happen.

	The treatments reflectance were predicted to respond as following relative to the control				
Prediction from:	Lo Cu	Hi Cu	Lo Zn	Hi Zn	Salt
Biology of tolerance	δ	δδ	δ	δδ	δ
Chlorophyll results	δ	δ	δ	δ	-

From the results, it was apparent that there was no pattern. An increase in reflectance at any particular wavelength in response to a low level of stress often did not correspond to a similar or increased reflectance with the high level of that stress. The results showed the responses of individual ecotypes to stress to be complex, and when different ecotypes were considered the variety of responses to the same stress revealed the difficulty of using remote sensing for detecting such stresses.

The only stress that elicited the same response in all ecotypes was salt, which caused a decrease in reflectance despite it not markedly affecting chlorophyll concentration. The effect of copper and zinc varied with dosage, ecotype and species. The variation in response between individuals in some cases was large, as shown where there was a large reflectance difference between averaged treatment reflectances and averaged control reflectances, but the results were not statistically significant. One area of possible interest was the relationship between the green and red spectral regions. In many treatments this area was flattened relative to the control largely through significantly decreased green reflectance. This was shown in these reflectance difference graphs as an increase in reflective difference between the green and red regions of the spectrum. An index was developed to highlight this feature in Chapter 5, Green Peak /Red Trough, (GP/RT). It's development is discussed in the next chapter.

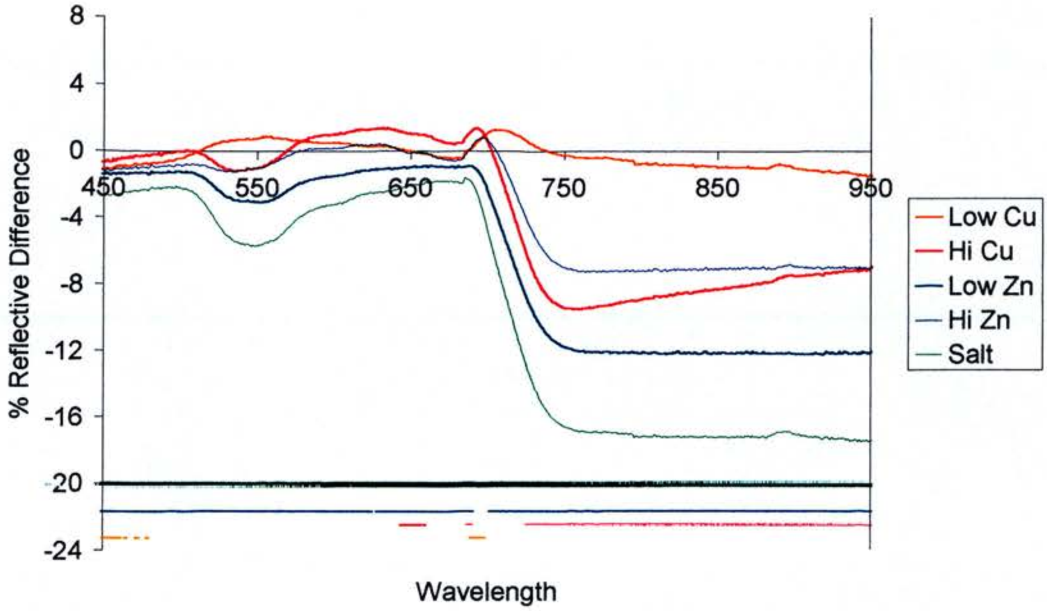

Figure 4.08. Reflective difference of *Ac* Coginan (T) treatments relative to the control. Lower lines (same colour coding) show significant Mann-Whitney U results between the control and that treatment.

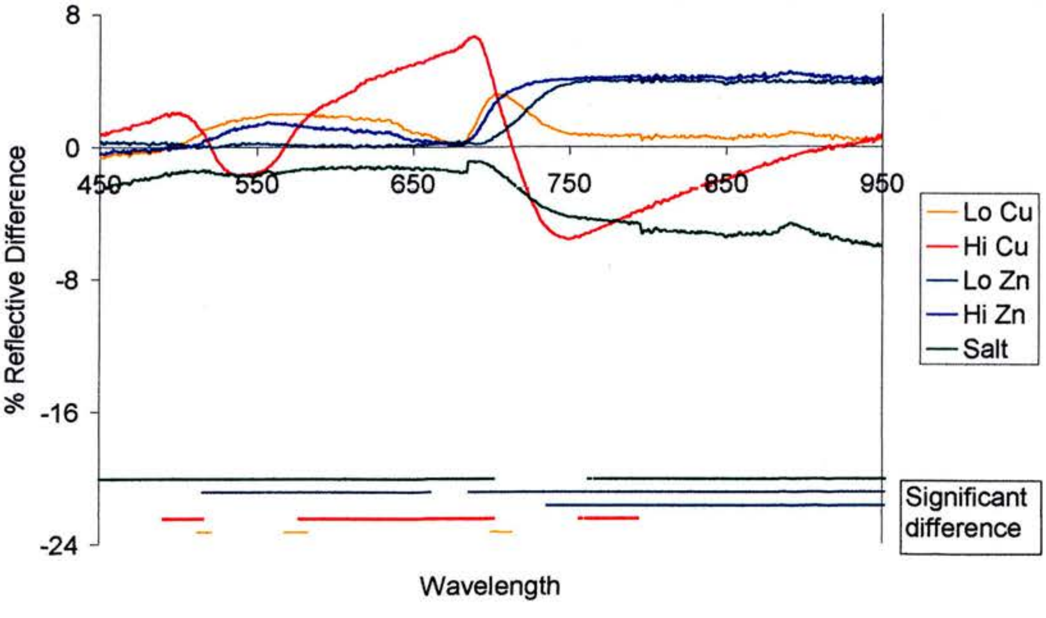

Figure 4.09. Reflective difference of *Ac* Lance (NT) treatments relative to the control. Lower lines (same colour coding) show significant Mann-Whitney U results between the control and that treatment.

4.3.4 Vegetation index results and discussion

The ideal index response should be linear or curvilinear, so that a higher dosage of metal changes the index value proportional to a lower dosage. Indices were tested here for the direction of response relative to control, and differences relative to the control were considered considering predictions based on biology and changes in chlorophyll concentration discussed earlier. The response to the same stress in different individuals/ecotypes/ species should ideally also be the same, although the response to different stresses may alter.

The results of the vegetation indices are presented in Tables 4.14 to 4.17. Each table shows the results of Mann-Whitney U tests for an ecotype comparing each treatment to the control. For example, Table 4.14's first row of results shows the results for *Fr* Jupiter (NT) comparing the results of the PSSRa index developed by (Blackburn, 1998b), across all treatments. Indices for the low copper, high copper, and high zinc treatments were all significantly different than the control, with the index results in all cases being lower than that of the control. The low zinc and salt treatments were not significantly different to the control.

Fr. Jupiter (NT) showed a significant response to all treatments in some of these indices (Table 4.14). A significant difference between the index results for control and high copper was found for all indices except Malthus 446/477. This and three others (Penuelas WBI, PRI and this study's GP/RT) did not discriminate between the low copper and control treatments. The direction of shift in index result relative to the control for those indices that show a difference for both copper treatments was the same in all except Penuelas NPCI.

All indices were able to distinguish between high zinc and control apart from two, Penuelas's WBI and PRI. Most indices did not distinguish the low zinc treatment from the control, with only six showing a significant difference. Three of those indices (Malthus 425/470, 818/713 and Hardy GP/RT) moved in a different direction than the high zinc treatment. The salt treatment, despite a wide range of wavelengths that showed a significant difference between itself and the control only had 6 indices with a significant response. The movement in all of these relative to the control was different to any significant high metal treatment, except using the GP/RT index. The only indices that distinguished between control and all treatments was Blackburns's PSSRb and PSNDa. The least effective index was Penuelas's PRI.

The predictions on what treatments should be identifiably different from the control based on knowledge of the biology of tolerance, and the chlorophyll results were given in Table 4.06. No indices matched the biology predictions exactly; Blackburn's PSNDa came closest, except the high treatment values were not distinct from the low. The chlorophyll predictions were matched by Blackburn's PSNDb. Malthus's 818/713 and 425/470 also matched the prediction, but the direction of response altered between the low and high zinc treatments.

For *Fr* Merlin (T) most indices distinguish the high copper treatment from the control, except some of Malthus's indices (Table 4.15). Only three indices distinguished more treatments, Blackburn's PSNDa, PSNDb and GP/RT, which distinguished the most treatments. PSNDa distinguished the high copper and zinc treatments from the controls, and PSNDb both copper treatments, although the direction of movement of the index was different. GP/RT distinguished all treatments from the control except low copper, and the direction of movement was the same in all. GP/RT was also the only index to respond to the salt treatment.

Predictions of what should be observed for *Fr* Merlin (T) were given in Table 4.05. Based on the biology it was predicted that one or both high treatments as well as salt should show a response, but neither low metal treatment should have affected index results. No indices matched the predictions based on biology, though Blackburn's PSNDa came close, with the only difference being that it did not respond to salt. This index did match the prediction based on chlorophyll results however, and no others did.

Comparing the significant high copper index results between *Fr*. Jupiter (NT) and Merlin (T) (i.e. comparing the non-tolerant (Table 4.14) to the tolerant ecotype (Table 4.15)) revealed that *Fr* Merlin (T) showed much less index responses to treatment, as would be expected for a tolerant ecotype. Where there was an index response in both ecotypes the direction of index movement relative to the control was generally the same. The exceptions were Penuelas's NPCI and this study's GPRT. Only one index, the GP/RT index, distinguished between the control and the same treatments in *Fr* Jupiter (NT) and *Fr* Merlin (T), although the direction of movement was not the same in all treatments. The best index in terms of conforming with the predictions for both ecotypes was Blackburn's PSNDa (Figure 4.10), although it moved in a different direction for the *Fr* Jupiter (NT) salt treatment. This index's response was similar in the other treatments, and there were fewer significant differences between control and treatments for *Fr* Merlin (T).

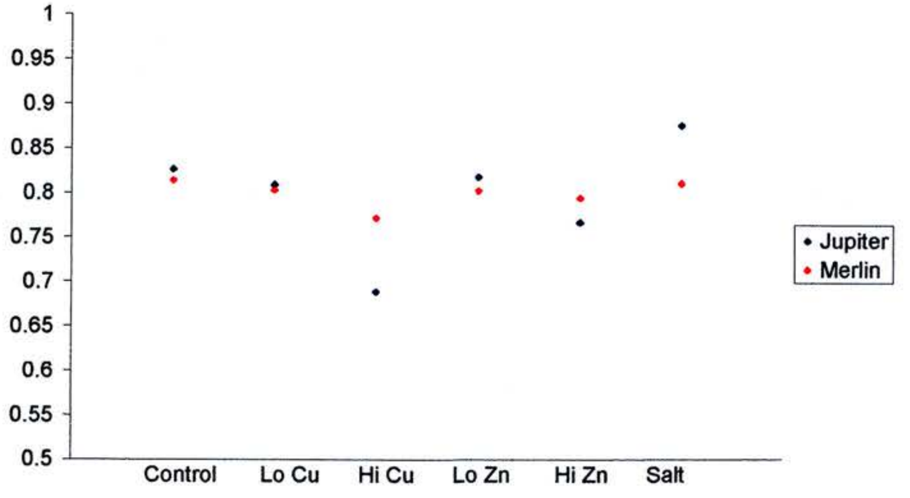

Figure 4.10. Average PSNDa index results for both *Festuca rubra* ecotypes for all treatments.

Table 4.14. Results of Mann-Whitney U tests comparing vegetation index results from treatments individually to the control for *Fr* Jupiter (NT). Arrows indicate a significant difference, with the index results for the treatment being lower (↓) or higher (↑) than the control, respectively. - indicates that there is no significant difference. See Appendix A, table A4.14 for statistical results.

			Comparison of treatment to control				
Author	Title	Index	Lo Cu	Hi Cu	Lo Zn	Hi Zn	Salt
Blackburn 1998b	PSSRa	800/675	↓	↓	-	↓	-
	PSSRb	800/650	↓	↓	↓	↓	↑
	ref. PSSRa	800/680	↓	↓	-	↓	-
	ref. PSSRb	800/635	↓	↓	-	↓	-
	PSSRc	800/500	↓	↓	-	↓	-
	PSNDa	(800-680)/ (800+680)	↓	↓	↓	↓	↑
	PSNDb	(800-635)/ (800+635)	↓	↓	↓	↓	-
Carter 1994		760/695	↓	↓	-	↓	-
Malthus et al 1995		425/470	↓	↓	↑	↓	-
		446/477	-	-	-	↑	↓
		541/836	↑	↑	-	↑	-
		818/538	↓	↓	-	↓	-
		818/713	↓	↓	↑	↓	-
Penuelas et al1994	WBI	970/900	-	↑	-	-	↓
	PRI	550-530/ 550+530	-	↓	-	-	-
	NPCI	680-430/ 680+430	↑	↓	-	↓	↑
Hardy	GP/RT	GP/RT	-	↓	↓	↑	↑

Table 4.15. Results of Mann-Whitney U tests comparing vegetation index results from treatments individually to the control for *Fr* Merlin (T). Arrows indicate a significant difference, with the index results for the treatment being lower (↓) or higher (↑) than the control respectively. "-" indicates that there is no significant difference. See Appendix A, table A4.15 for statistical results.

			Comparing Control to these treatments individually using MW-U test				
Author	Title	Index	Lo Cu	Hi Cu	Lo Zn	Hi Zn	Salt
Blackburn 1998	PSSRa	800/675	-	↓	-	-	-
	PSSRb	800/650	-	↓	-	-	-
	ref. PSSRa	800/680	-	↓	-	-	-
	ref. PSSRb	800/635	-	↓	-	-	-
	PSSRc	800/500	-	↓	-	-	-
	PSNDa	(800-680)/ (800+680)	-	↓	-	↓	-
	PSNDb	(800-635)/ (800+635)	↑	↓	-	-	-
Carter 1994		760/695	-	↓	-	-	-
Malthus et 1995		425/470	-	-	-	-	-
		446/477	-	-	-	-	-
		541/836	-	-	-	-	-
		818/538	-	-	-	-	-
		818/713	-	↓	-	-	-
Penuelas et 1994	WBI	970/900	-	↑	-	-	-
	PRI	550-530/ 550+530	-	↓	-	-	-
	NPCI	680-430/ 680+430	-	↑	-	-	-
Hardy	GP/RT	GP/RT	-	↓	↓	↓	↓

Table 4.16. Results of Mann-Whitney U tests comparing vegetation index results from treatments individually to the control for *Ac* Coginan (T). Arrows indicate a significant difference, with the index results for the treatment being lower (↓) or higher (↑) than the control respectively. - indicates that there is no significant difference. See Appendix A, table A4.16 for statistical results.

			Comparing Control to these treatments individually using MW-U test				
Author	Title	Index	Lo Cu	Hi Cu	Lo Zn	Hi Zn	Salt
Blackburn 1998	PSSRa	800/675	-	↓	↓	-	-
	PSSRb	800/650	-	↓	↓	↓	↓
	ref. PSSRa	800/680	-	↓	↓	-	-
	ref. PSSRb	800/635	-	↓	↓	-	-
	PSSRc	800/500	-	↓	↓	↓	↓
	PSNDa	(800-680)/ (800+680)	-	↓	↓	-	-
	PSNDb	(800-635)/ (800+635)	-	↓	↓	↓	↓
Carter 1994		760/695	-	↓	↓	↓	↓
Malthus et 1995		425/470	↓	↓	-	↓	↓
		446/477	↓	↓	-	-	↓
		541/836	-	↑	↑	↑	↑
		818/538	-	↓	↓	↓	↓
		818/713	↓	↓	↓	↓	↓
Penuelas et 1994	WBI	970/900	↓	-	-	-	↓
	PRI	550-530/ 550+530	-	↑	-	↑	-
	NPCI	680-430/ 680+430	↑	↑	↑	↑	↑
Hardy	GP/RT	GP/RT	↑	↓	-	-	-

Table 4.17. Results of Mann-Whitney U tests comparing vegetation index results from treatments individually to the control for *Ac* Lance (NT). Arrows indicate a significant difference, with the index results for the treatment being lower (↓)or higher (↑) than the control respectively. - indicates that there is no significant difference. See Appendix A, table A4.17 for statistical results.

			Comparing Control to these treatments individually using MW-U test				
Author	Title	Index	Lo Cu	Hi Cu	Lo Zn	Hi Zn	Salt
Blackburn 1998	PSSRa	800/675	-	↓	-	-	↑
	PSSRb	800/650	↓	↓	↑	-	-
	ref. PSSRa	800/680	-	↓	-	↑	↑
	ref. PSSRb	800/635	-	↓	-	↑	↑
	PSSRc	800/500	↓	↓	-	↓	-
	PSNDa	(800-680)/ (800+680)	-	↓	-	-	↑
	PSNDb	(800-635)/ (800+635)	↓	↓	-	-	-
Carter 1994		760/695	↓	↓	-	-	-
Malthus et 1995		425/470	↓	↓	-	-	↓
		446/477	-	↓	-	-	↓
		541/836	↑	↓	↓	-	-
		818/538	↓	-	↑	-	-
		818/713	↓	↓	-	-	↓
Penuelas et 1994	WBI	970/900	-	↑	-	-	↓
	PRI	550-530/ 550+530	↑	-	↑	↑	↑
	NPCI	680-430/ 680+430	↑	↑	↓	-	↑
Hardy	GP/RT	GP/RT	-	↓	-	↑	↑

The index results for *Ac.* Coginan (T) showed the most orderly index results: if an index had a significant result for two or more treatments, the direction of each value relative to the control was the same except for one index, GP/RT (Table 4.16). The high copper treatment showed a significant response using all indices except Penuelas's WBI. The low copper treatment only showed a significant response in six indices, one of which shows a significant response to low copper but not to high copper (Penuelas WBI). For the zinc treatments more indices showed a response for the low than for the high treatment. Malthus 446/477, Penuelas's WBI and GP/RT showed no response to either zinc treatment. Salt evinced a significant response in all except 6 indices.

The indices that distinguished between the control and all treatments were Malthus 818/713 and Penuelas NPCI. No indices matched the biology predictions, although Penuelas's PRI was closest(Table 4.07). No indices matched the chlorophyll predictions. All indices that came closes to matching the predicted responses based on chlorophyll results also responded to salt, which was not predicted.

For *Ac* Lance (NT) most indices showed a response for high copper except for Malthus 818/538 and Penuelas PRI, both of which did show a significant response for low copper (Table 4.17). Ten indices showed a significant response for both copper treatments, only one of which moves in a different direction for each (Malthus 541/836). Much fewer indices responded significantly to the zinc treatments and only one responded to both (Penuelas's PRI). Twelve indices responded to the salt treatment, generally in a different direction to any metal response for that index. No indices matched the prediction based on biology or chlorophyll content (Table 4.08). The indices that showed a response to most treatments were Penuelas's PRI and NPCI, although the PRI did not distinguish between the control and high copper treatment, and the NPCI did not respond in a constant direction, and showed no response to high zinc.

Comparing between *Ac.* Coginan (T) and *Ac* Lance (NT) index results it was not immediately obvious which ecotype was the tolerant one, although it was known that *Ac* Coginan (T) was tolerant. Whilst index behaviour was as might be expected for Cu (less response in *Ac* Coginan (T)) the opposite was the case for Zn (greater response for *Ac* Coginan (T)). No index matched the predictions made based on biology or chlorophyll content. Penuelas's PRI indices gave results most similar to these predictions, although it could not distinguish the high copper treatment from the control for *Ac* Lance (NT).

Across both species and all ecotypes only Blackburn's reformed PSSRb (ref. PSSRb) and Carter's 760/695 indices responded to all stresses by moving in the same direction. Ref. PSSRb could differentiate between the control and 11 treatments, and Carter's index between 9 (of a possible total of 20). Penuelas's NPCI differentiated between more treatments, but did so in different directions for different ecotype/treatment combinations. Blackburn's PSNDa conformed best with predictions for *Festuca rubra*, while Penuelas' PRI conformed best for *Agrostis capillaris*. The complexity of ecotypes responses to treatments is shown by the variety of index results given here.

Comparing the results between the two non-tolerant ecotypes which were expected to have shown significant differences at all levels of treatment, only one index responded in the same way for both ecotypes in both high treatments, Blackburn's PSSRc. Malthus's 425/470, and 818/713 responded to both low and high treatments in the same direction for both ecotypes in response to copper. No index was as effective for both of the zinc treatments. The GP/RT responded in the same way for both ecotypes in response to salt, and was the only index to do so. For detecting high levels of stress in both non-tolerant ecotypes Blackburn's PSSRc was therefore the most successful index, with no index detecting all levels of stress.

No index responded to both high metal treatments in the same direction for both tolerant ecotypes. Blackburn's PSNDa performed closest to this ideal, although it showed no response to high zinc for *Ac* Coginan (T).

The average of the index values obtained for the Carter's 760/695 index is given in Figure 4.11. This showed the difficulty of using these indices in the natural environment. Within each species significant results were lower for the treatments than control, but the values overlapped between ecotypes making the location of stressed areas without detailed plant community data impossible. Assuming an unknown community comprising only one of the 4 ecotypes, if the index result was less than 3, the community could be identified as being stressed. Any value between 3 and 5 could indicate a stressed or non-stressed environment, depending on which ecotypes were present, and a value of greater than 6 would have meant no stress.

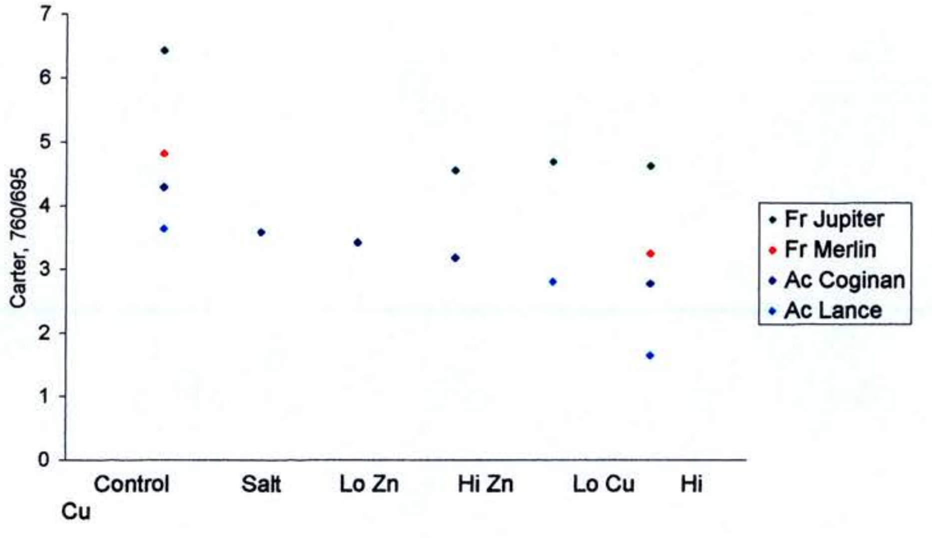

Figure 4.11. Response of Carter's 760/695 index to the different stresses for all ecotypes. Only results for treatments significantly different from the control were shown.

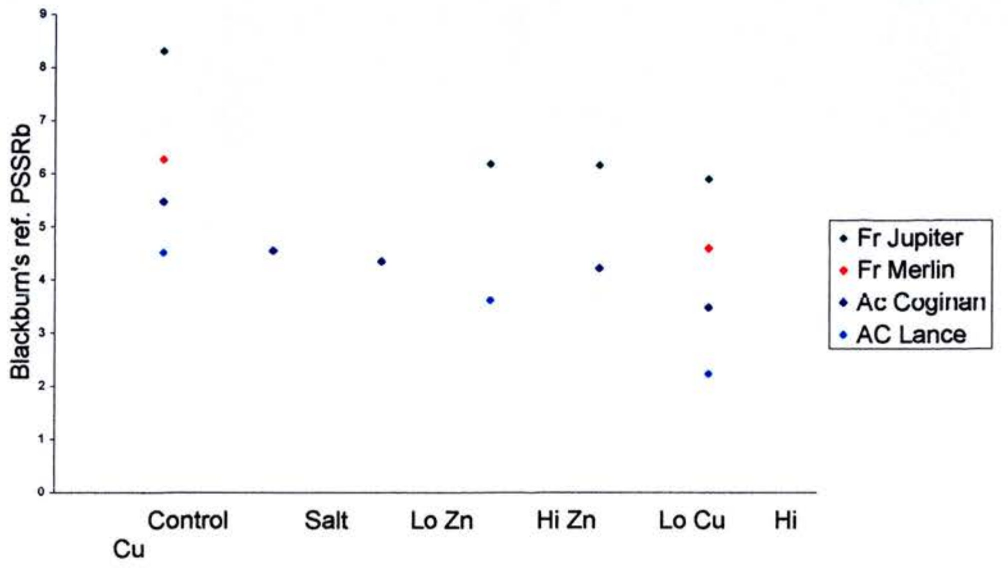

Figure 4.12. Response of Blackburns reformed PSSRb index to the different stresses for all ecotypes. Only results for treatments significantly different from the control were shown.

A similar confusion would occur using Blackburn's ref. PSSRb index (Figure 4.12). Values less than four would indicate a stressed community, and greater than 6.5 an unstressed one, but any value in-between could represent either contaminated or uncontaminated soil, depending on the ecotypes present.

Indices which did not provide such good results, those that move in either direction with different stresses or species gave more confusing results (Figure 4.13). The GP/RT index showed many significant differences between treatments (13 of a possible 20), but 5 were higher than the control and 8 lower. If this index was used to identify stressed areas within a unknown community comprised of one of the four ecotypes all that could be stated about the community was that, if the index was less than 2, the community was stressed. Other index values could represent a stressed or unstressed condition, showing why a constant direction of response for an index is desired.

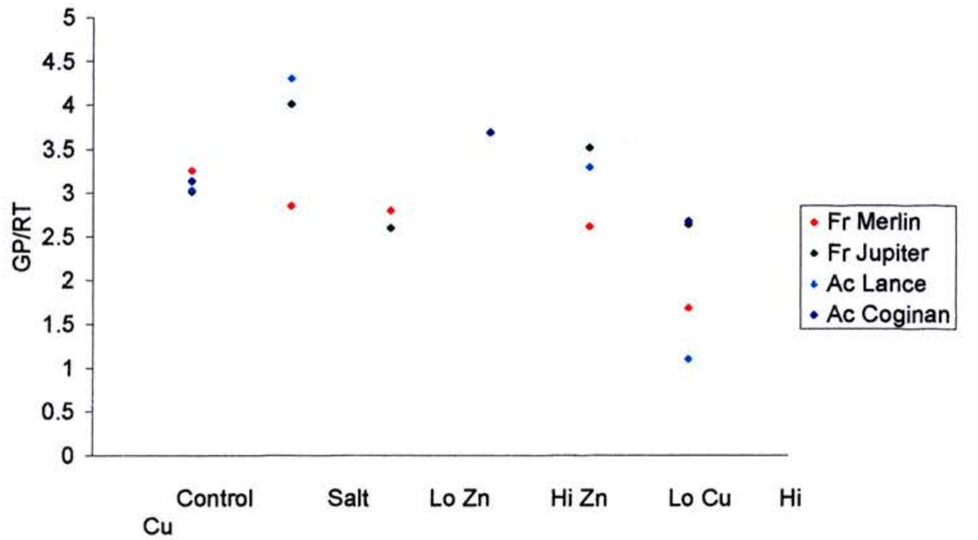

Figure 4.13. Response of GP/RT index to the different stresses for all ecotypes. Only results for treatments significantly different from the control were shown.

Not only was a great deal of information required about the species present and having a known control area, as some previous studies have intimated (Clark *et al.*, 1995; Wagner and Howarth, 1989; Birnie and Francica, 1981), but information was also required about the ecotype. No study has previously considered this, but these results show that it has a great deal of influence on the response of an index. The response was so different in many cases it could have been interpreted as that of a different species.

4.3.5 Red edge position

The changes of the Red Edge Position (REP) with treatment on the ecotypes is shown in Figures 4.14 and 4.15, and significant differences between the control and treatments were given in Table 4.18.

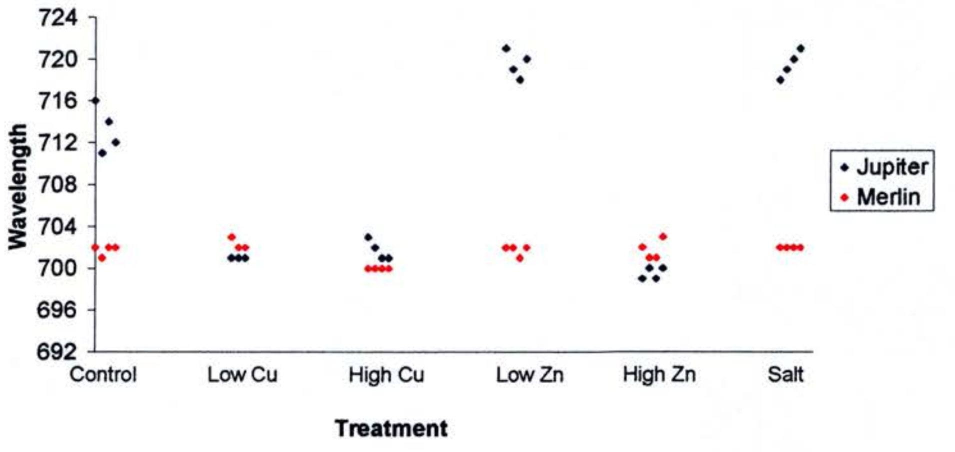

Figure 4.14. The response of Red Edge Position to treatment for both ecotypes of *Festuca rubra.*

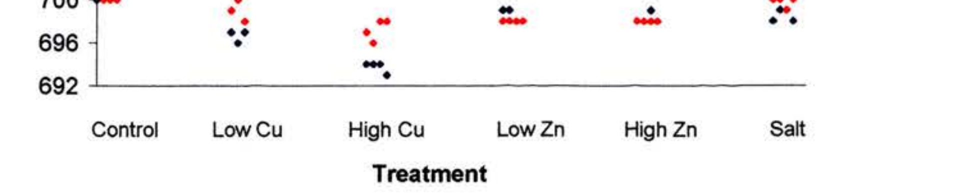

Figure 4.15. The response of Red Edge Position to treatment for both ecotypes of *Agrostis capillaris.*

Fr Jupiter's (NT) REP moved to shorter wavelengths relative to the control in both copper treatments, and the high zinc treatment. Neither the low zinc nor salt stress affect the REP (Figure 4.14), so neither prediction (Table 4.06) was met.

The tolerant ecotype (*Fr* Merlin (T)) showed a small though significant response to high copper. This matched one possible prediction based on biology (Table 4.05), that the ecotype was tolerant to all levels of treatment, except high copper. However, the salt treatment did not respond. The predictions based on chlorophyll results were not borne out by the REP, the high copper treatment responded, but the high zinc REP did not.

It was expected that the tolerant ecotypes REP for low level treatments would be the same as the non-tolerant's control REP as they would be unstressed. Instead *Fr* Merlin (T)'s REP for all treatments was indistinguishable from the *Fr* Jupiter (NT) REP in high zinc and both copper treatments. The variation in control REP within a species would make interpretation of REP results difficult in the field.

For both *Agrostis capillaris* ecotypes the REP in all treatments was very similar (Figure 4.15). *Ac* Lance's (NT) REP showed a small response (Fig. 4.15) to all treatments except low zinc. This was different to both of the predicted effects (Table 4.08). *Ac* Coginan's (T) REP showed very slight movement, and was the only ecotype to match the prediction based on chlorophyll concentration (Table 4.07). The control REP's of both ecotypes were very similar, with only very small shifts in REP differentiating between treatments.

If there were an unknown community consisting of only on of these ecotypes all that could be inferred from REP would be that if REP was at less than 700 nm the community is on contaminated ground, and if it was at wavelengths greater than 700 nm the community could be on either contaminated or uncontaminated ground depending on the ecotype. 700 nm would normally be interpreted by most users as being an indicator of stressed plants, and contamination would be inferred (e.g. (Jago *et al.*, 1999)).

Table 4.18. Results of Mann-Whitney U tests comparing Red Edge Position results from treatments individually compared to the control for each ecotype. ↓ indicates a significant difference, with the REP for the ecotype being lower than the control.

- indicates that there was no significant difference.

	Comparing Control to these treatments individually using MW-U test				
Species cultivar	Lo Cu	Hi Cu	Lo Zn	Hi Zn	Salt
Fr Jupiter (NT)	↓	↓	-	↓	-
Fr Merlin (T)	-	↓	-	-	-
Ac Coginan (T)	-	↓	↓	↓	-
Ac Lance (NT)	↓	↓	-	↓	↓

4.4 Conclusions

The theory behind using remote sensing of vegetation to detect contaminated soil is that a stress response to the soil contamination is identifiable using remote sensing through changes in the vegetation reflectance. A decline in chlorophyll concentration has been chosen by many studies (Carter *et al.*, 1996; Blackburn, 1998a) as such a stress response. The results shown here for pigment analysis show how this methodology is flawed if tolerant ecotypes are considered (Fig. 4.05). If biochemical analysis of these plants was used to identify contaminated ground the interpretation would vary widely with what ecotype was sampled. Low levels (<5 mg g^{-1} Chl. *a* dry wt) would indicate presence of metal, although the level of contamination or the identity of metal could not be obtained. Higher chlorophyll levels could indicate non-contaminated areas, or contaminated areas if tolerant plants are sampled. Remote sensing, as a less direct measure of chlorophyll, can be expected to have greater difficulties.

Hypothesis 1 was concerned with the presence of a stress effect, ignoring the existence of tolerance (tested here just between the two non-tolerant ecotypes). These results showed an effect of stress on non-tolerant plants, although an identifiable and generalisable stress detection method was not found, i.e. neither reflectance analysis, vegetation indices or red edge position could be used to confirm the presence of all stress treatments regardless of species. The reflectance data themselves were confused. One vegetation index could be used to distinguish high metal treatments from the control in both species (PSSRc). If the contaminant identity was known five indices worked in both species to identify copper stress (PSSRb, PSSRc, Carter's, Malthus 425/470 and 818/713), although none worked for both zinc treatments. Four indices could be used for locating salt contamination, again assuming that the plants were known to be non tolerant and the identity of the stressor was known (PSNDa, Malthus 541/836 , WBI and GP/RT). The REP could not be used to identify stress due to *Ac* Lance's (NT) REP being very similar to it's stress treatments as well as *Fr* Jupiter's (NT) stress REP's. In the case of non-tolerant ecotypes, if the species and possible contaminants are known then the indices and REP are more useful and could be used to show stress effects. This is a very high requirement for prior knowledge, however, and negates remote sensing usefulness as an investigational technique.

Introducing tolerant ecotypes (and testing Hypothesis 2) introduces another layer of complexity. The tolerant ecotypes didn't respond exactly as predicted, and did not explain all the results. Some results were different regardless of tolerance; that ecotype responded differently to the non-tolerant one, but not as would be predicted based on tolerance (e.g. the tolerant *Agrostis capillaris* ecotype responded to more indices under zinc contamination than did the non-tolerant ecotype). It would be interesting to compare the reflectance properties of two non-tolerant ecotypes of the same species to see how they differ. These results would suggest that for some reflectance parameters (e.g. REP for *Festuca rubra* control measurements) the tolerant ecotype responses are different enough to be mistaken for a different species to the non-tolerant ecotypes.

Clark *et al.* (1995) stated that a library of species spectral responses at different stages of their phenology would be beneficial to remote sensing studies. This study indicates that species reflectance varies even within species, at the level of the ecotype. This would suggest that a library of ecotype responses is not a viable proposition, and thus the viability of remote sensing for identifying stress responses in plants, and in turn relating those to the presence of metal contamination is called into question. Furthermore, while a botanist could distinguish different species, growth experiments or genetic analysis would be required to identify different ecotypes of the same species.

Leaf spectral parameters are only a small part of remote sensing measurements made in the field, where topography and canopy characteristics influence reflectance. In the next chapter canopy reflectance of the two *Festuca rubra* ecotypes under zinc stress is investigated.

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Chapter 5: Remote Sensing of Canopy Reflectance

5.1 Introduction

This chapter investigates the canopy reflectance spectral response of two ecotypes (one tolerant (T), one non-tolerant (NT)) of the grass, *Festuca rubra* (*Fr*), under different levels of contamination. The two cultivars investigated were *Fr* Jupiter (NT) and *Fr* Merlin (T). These were grown at different levels of zinc contamination (control, low and high). The plants were grown in monoculture and in a 50:50 (NT:T) mixture. Remote sensing measurements were investigated as reflectance, vegetation indices and red edge position (REP). These effects were related to treatment and the chlorophyll concentration of sampled leaves. This research had an experimental design consisting of plots of standardised soil grown outside, with control and metal applied treatments.

The following hypotheses were investigated:

Hypothesis 1: *The canopy spectral response of non tolerant grass on clean soil differs from the same grass on contaminated soil.*

Other studies have investigated the spectral response of plant species to stress, though none have mentioned the tolerance of the species under investigation. Changes in the value of a vegetation index or REP have been cited as an indicator of stress.

Hypothesis 2: *The canopy spectral response of tolerant grass* ***does not*** *alter with levels of contamination that affects the spectral response of non-tolerant grass.*

No other studies have investigated this; the occurrence of tolerant plants has been ignored.

These hypotheses are based on the following assumptions:

Assumption 1: The cultivars only differ in response to metal, and are indistinct in every other respect.
The biology of ecotypes suggests that there will be some differences between ecotypes other than just tolerance. However, this is likely to be slight and the experimental design will control for it.

Assumption 2: Stress is caused by the metal.
The experimental technique means that the only variable being altered between treatments is the level of metal contamination.

5.2 Method

5.2.1 Choice of test organism.

The study had a randomised block experimental structure, with different metal treatments of grass grown on artificial soil plots. The biological requirements for the study were two ecotypes of a species that differed only in tolerance to a metal. Ecotypes of a species generally differ in more than this, e.g. a tolerant ecotype may also be smaller. The only tolerant ecotype in commercial supply was *Festuca rubra* Merlin (*Fr* Merlin (T)). A commercial supply was necessary to provide enough material of known standard to give a full canopy coverage on replicate plots. *Fr* Merlin (T) is tolerant to high levels of zinc, lead and copper ((Davies *et al.*, 1991); (Powell *et al.*, 1986)), and was sourced from Johnson's Seeds (London Road, Boston, Lincolnshire, PE21 8AD) along with a non-tolerant *Festuca rubra* Jupiter (*Fr* Jupiter (NT)) ecotype.

5.2.2 Plot set-up

Boxes were constructed to be 75 cm * 75 cm * 20 cm deep (approx. 140 litres in volume) using untreated pine timber (Figure 5.01). The bases were slatted to allow free drainage, as water-logging of the soil was considered to be a potential problem. Water-logging and its associated plant stress was also deemed more difficult to solve than drought if it occurred mid-experiment, which could be easily rectified by additional watering. The boxes were lined with a fine cheese-cloth to allow free drainage yet retain soil. Each box was raised two inches above ground level to further aid drainage on 2" x 4" timbers. The boxes were laid out on black plastic sheeting to inhibit weed growth around the plots, and invasion by weeds onto the plots.

The soil in the box was made up to a standard ecotoxicology mixture (J. Weeks, pers. comm., Table 5.01). The soil was mixed in a clean cement mixer to ensure an even composition. The first few mixes were checked for pH, and adjusted with $CaCO_3$ as necessary to between pH 6-7. The following mixes had the same amount of $CaCO_3$ added.

Table 5.01. Constituents used as the basis for the artificial soil on which grass treatments were grown.

Ingredient	Percentage by dry weight	Percentage by volume (approx.)
Peat – Finely ground	10%	30%
Clay – Fine marl	20%	20%
Sand – Fine	70%	50%

The plots were watered evenly as required via a mains hosepipe. Care was taken to direct the spray of water to simulate a rain effect, so the seeds/seedlings were not disturbed. With the high proportion of sand in the boxes it was necessary to water daily during sunny periods. Surface dampness was assessed in deciding the necessity of watering, and all plots were watered at the same rate for approximately the same length of time to ensure equivalence between plots. Watering was generally performed in the evening to provide maximum water retention with minimum evaporation and salt formation.

Two experiments were designed, a monoculture canopy experiment in 2000, and a mixed canopy experiment in 1999. Despite the chronological sequence of experiments, the monoculture is discussed first, followed by the mixture experiment.

5.2.3 Monoculture experiment

The soil was added to the boxes which had been laid out in a randomised block design. The monoculture experiment had 5 blocks of 6 treatments (Figure 5.02), taking care to avoid placing them too close to the hedge or trees to avoid shading. Seed was sown on to the plots at a rate of 10 g m^{-2} on 29/4/2000. The seed germinated within 2 weeks, and had a high survivorship.

Due to a slow growth rate it was necessary to promote growth, and the plots were fertilised on 24/5, 7/6, 21/6, 5/7 (High N), and 12/7 (High N). For the first three applications Miracle-Gro™ all-purpose plant food, N15, P30, K15 (Nitrogen, Phosphorous, Potassium) was added at the recommended dose. This appeared to have negligible effect, so high nitrogen lawn food was applied on the final two dates (N30, P30, K15). It was considered that this was required due to low nutrient levels because of the low fertility substrate and a very low level of microbial activity (so little release of nutrients) in the plots. The benefit of a lack of microbial activity is that different metal treatments did not affect nutrient cycling, so results were from a direct effect of metal content on plant physiology. During the growth of the plots weeding was carried out, but was only required on a limited basis.

Following establishment of a full canopy cover zinc was added to the treatment boxes as $ZnSO_4.7(H_2O)$ as a solution through a fine watering rose providing 150 μg g^{-1} dry weight of soil per application (high zinc plots) and 75 $\mu g\ g^{-1}$ (low zinc plots). This was added on 14/8, 8/9, and 29/9. By the end of the experiment the total zinc received by the high zinc plots was 450 $\mu g\ g^{-1}$ and the low zinc plots 225 $\mu g\ g^{-1}$ (treatment codes in Table 5.02). This value is not necessarily the same as the total zinc in the plots at the end of the experiment because of leaching and/or uptake. The values are equivalent to concentrations listed in the literature (ICRCL, 1987; Ross, 1994) that induce stress.

Table 5.02 Treatment codes used in this chapter

Ecotype	Treatment	Code
Fr Jupiter (NT)	Control	JC
	Low Zinc	JL
	High Zinc	JH
Fr Merlin (T)	Control	MC
	Low Zinc	ML
	High Zinc	MH

Fr Jupiter (NT) is the non-tolerant cultivar, while *Fr* Merlin (T) is the tolerant cultivar.

5.2.4 Mixture experiment

The two ecotypes seed was fully mixed and sown onto the plots at a rate of 10 g m^2 on 20/6/1999. The plots were fertilised with Miracle-Gro^{TM} all-purpose plant food , N15, P30, K15 at the recommended dose on 30/7, and 15/8. Following establishment of a full canopy cover zinc ($ZnSO_4.7(H_20)$, was added as a solution through a fine watering rose at levels of 150 $\mu g\ g^{-1}$ dry weight of soil per application (high zinc plots) and 75 $\mu g\ g^{-1}$ (low zinc plots). This was added on 2/9, and 2/10. By the end of the experiment the total zinc received by the high zinc plots was 300 $\mu g\ g^{-1}$ and the low zinc plots 150 $\mu g\ g^{-1}$.

5.2.5 Biochemical Analysis

Biochemical analysis of the plant material was carried out during the experiments. Chlorophyll analysis was determined by acetone extraction and subsequent spectrophotometer measurements following the methods of (Sestak *et al.*, 1971). Only the second leaves were selected to ensure that all leaves were at the same stage of growth so that pigment concentration was related to stress, and fluctuations were not caused by differing ages of leaves. The leaves were stored in a dark, cold area and transported to the laboratory to be analysed the same day. The leaves from the plots were sorted so that each replicate was approximately 0.2 g per sample. The leaves were weighed, ground with a pinch of washed sand and $MgCO_3$ (to neutralise any acids) with approximately 5 ml 80% acetone in a mortar and pestle. After around 3 minutes vigorous grinding, when there were no more visible pieces of grass, the contents of the mortar were washed into a graduated centrifuge tube. The mortar and pestle were then rinsed with more 80% acetone so that the volume in the tube was no more than 10 ml, and there was no more residue in the mortar or on the pestle. The tube was then spun at 3000 rpm for 5 minutes in a centrifuge to precipitate all solid matter at the bottom of the tube and ensure an uncontaminated liquid sample. The tubes were topped up to 10 ml as necessary with acetone (80%) and as much of the supernatant as necessary decanted into a 1 cm^2 cuvette. This was then placed in the dual beam spectrophotometer (Perkin-Elmer Lamda 40 UV/Vis), which had previously been zeroed across all wavelengths with a pair of cuvettes containing only 80% acetone. A full scan of transmittance from 400-1100 nm was undertaken at 1 nm resolution. The concentration of chlorophyll was calculated as in (Lichtenthaler, 1987), as mg g^{-1} fresh weight of the leaf.

5.2.6 Reflectance measurements

Reflectance measurements were carried out using a moveable outside light-proof environment (tent) with artificial lighting inside. This enabled measurements to be made of each plot with a constant level of illumination on a single day and over time. The frame was made of Dexion 140 (steel girders, painted matt black). The frame was 215 cm high, and 85 cm square. The spectroradiometer was mounted 150 cm above the ground, 125 cm above the soil surface of the plots, and generally 115 cm above the grass canopy surface (Figures 5.03, 5.04). Two 1000W video lights were mounted as shown, with power supplied by an armoured mains cable from a source with no other power requirements so power surges and their associated effects on light output were avoided. The electrical system included a circuit breaker. The frame was covered with a black cotton cloth to create a matt inner, along with a black polyethylene pond liner outer to ensure no light transmission from outside (Figures 5.05, 5.06). With the electrical equipment being used outside, measurements could only be carried out under dry conditions with no wind.

A GER3700 spectroradiometer on loan from the Natural Environmental Research Council Equipment Pool for Field Spectroscopy (NERC-EPFS) was used for remote sensing measurements. The specifications of the GER3700 are given in Table 5.03. This is a high spectral resolution instrument with measurements extending from the visible region to well into the middle infra red. The spectroradiometer was mounted in the tent so that its lens was central in the frame. The lens was approximately 115 cm above the grass canopy, so that the field of view using the 10 degree optic had a diameter of approximately 20 cm. The reflectance measurements were standardised against the reflectance of a calibrated white Spectralon™ reference panel. The Spectralon™ reference panel was approximately 25 cm square, so use of the laser sighting (accounting for the laser's offset) enabled accurate reference panel measurements. The panel was supported using temporary mounts on the lower cross struts of the frame above the canopy thus avoiding canopy disturbance. The spectroradiometer and the laptop computer needed for its operation were powered by external 12V battery packs. The GER3700 was operated by first taking a reference scan of the Spectralon™ panel. Then target scans were taken, which were then normalised against the reference scan to create % Reflectance (using NERC-EPFS RefG3700 software). The individual spectra were based on an average of 16 scans taken by the GER3700. New reference scans were taken over every plot so any variation in the irradiance conditions was accounted for.

Table 5.03. Characteristics of the spectroradiometer (GER 3700).

Character	Characteristics
Spectral Range	350nm – 2500nm
Bandwidth* – 350 nm – 1050 nm	1.5 nm
1050 nm – 1850 nm	6.5 nm
1850 nm – 2500 nm	9.5 nm
Field of view used (f.o.v.)	10 degree
Sighting	Laser

*The data were interpolated to 1nm bandwidths using NERC-EPFS RefG3700 software.

Experiments were carried out to characterise the data collection design used. Experiment 1 investigated the response of any heating effect on the spectral output of the lights over time (Table 5.04). The Spectralon™ reference panel was used as both the reference (at time 0) and target (0 - 30 min) and it was assumed it's reflectance would be unaffected by any heat from the lights. Measurements of the panel were made at one minute intervals for 30 minutes. In all experiments the lamps were warmed up beforehand for 30 minutes, as this is the most likely time for heating to alter the lapms output spectrum (pers. comm. D. Emery, NERC EPFS co-ordinator).

Table 5.04. Experiment 1. Effect of lights warming up on their spectral output.

Order	Action
1	Lights turned on and allowed to warm for 30 minutes
2	GER3700 mounted onto frame, covers placed over frame
3	Reference panel situated in field of view of spectroradiometer
4	Target and reference measures made of panel
5	Lights kept on, covers in place, measurements taken every minute for 30 minutes

Experiment 2 (methods in Table 5.05) investigated the response of a green standard (dry green nylon scrubbing pads) to estimate a signal to noise ratio for the tent and artificial lighting. The pads were recommended as a target as they were equivalent to a rough grass canopy and unaffected by moisture levels or any heating by the lamps (pers. comm. D. Emery). The pad reflectance should thus be identical over sequential measurements.

Table 5.05. Experiment 2. Signal - Noise relationship for the set up using nylon scrub pads.

Order	Action
1	Lights turned on and allowed to warm up for 30 minutes
2	Covers placed over frame, reference panel placed in f.o.v. and reference measure taken, reference panel removed
3	Scrub pads arranged in f.o.v. of instrument, target measures taken sequentially as close to each other as possible for up to 5 minutes to simulate the time required for taking measures over the grass plots.

The methods used in data collection over the grass plots themselves are given in Table 5.06. This regime ensured that any change in technique was minimised and would not preferentially affect one treatment more than the others. For the monoculture experiment remote sensing measurements were carried out on four days: 25 August, 5 September, 16 September and 15 October 2000. For the mixture experiment remote sensing measurements were made on 7 October, 22 October and 16 November 1999. The 7 October data has been discarded as rain interrupted the complete measurement of all plots. These measurements were made late in the season because the slow growth of the canopies meant that they were not at full cover until these times. Dates were selected based on dryness of the plots (as dry as possible), weather conditions and the availability of the GER3700 from the NERC-EPFS. One remote sensing measurement was taken of the centre of each plot on each date.

Table 5.06. Spectral data collection techniques used over the grass treatments.

Order	Action
1	Warm up lights for 30 minutes
2	Move frame over plot (plots scanned in no particular order within blocks, blocks chosen sequentially around site).
3	Apply covers
4	Insert reference panel over plot, using laser sighting to centre
5	Take reference measurement, ensuring covers are closed
6	Remove panel, re-fit covers and take target measure
7	Remove covers
8	Allow spectroradiometer to cool (casing at ambient temperatures). Repeat from stage 2

5.3 Data Analysis

The remote sensing data were calibrated to percentage reflectance (reflectance or R_{λ}) and interpolated to 1 nm using NERC-EPFS software. Data are presented as averaged reflectance unless otherwise stated. Variation within replicates was calculated as the average reflectance for each treatment divided by the standard deviation for each treatment (Avg/Stdev). A high Avg/StDev means that the variation in reflectance between replicates for that treatment was low (i.e. they all showed similar reflectance values). Data are also presented as the reflective difference, which is the difference between the control and the metal treatments in percentage reflectance units at each wavelength (Carter *et al.*, 1992). This is a technique to better highlight the differences between treatments and the control, for example, a reading at any wavelength for the high zinc treatment of "1" means that at that wavelength, that treatment is 1 percentage reflectance unit higher than the control. The control itself can be considered to be zero. Reflectance was analysed statistically to determine if treatments were significantly different at each wavelength. The results were first considered at each separate date (i.e. are the treatments of the same cultivar different?), and later for each treatment over different dates (i.e. does the treatment reflectance change over time?).

The first derivative of reflectance was also used, which allowed for the comparison of the shape of the spectral curves, and is considered here between 500 and 1000 nm. First derivative results were obtained using least squares polynomial first order smoothing, with a smoothing width of 11 (Savitzky and Golay, 1964). The smoothing width was determined qualitatively as being the minimum width to identify the signal, and the maximum width to suppress noise. The Red Edge Position (REP) is located in this study as being the wavelength of the peak of the first derivative at around 710 nm.

5.3.1 Indices

Vegetation indices were selected from the literature, and developed as part of this study (Table 5.07). The indices chosen from the literature were developed to either measure stress or pigment concentrations, or are commonly used general vegetation indices (e.g. NDVI, SAVI). Indices were also developed during this study, and the factors concerning their development are discussed with the data set that contributed to their development.

Table 5.07. Vegetation indices gathered from the literature used in this study.

Author	Name of Index	Formulae	Application
(Blackburn, 1998)	PSSRa	800/675	chlorophyll a
	PSSRb	800/650	chlorophyll b
	reformed PSSRa	800/680	chlorophyll a
	reformed PSSRb	800/635	chlorophyll b
	PSSRc	800/500	carotenoids
	PSNDa	$\frac{800-680}{800+680}$	chlorophyll a
	PSNDb	$\frac{800-635}{800+635}$	chlorophyll b
(Carter and Miller, 1994)		695/760	
(Malthus *et al.*, 1995)		425/470	stress
		446/477	stress
		541/836	stress
		818/538	stress
		818/713	stress
(Dawson *et al.*, 1999)		NDVI SAVI	vegetation amount vegetation amount
(Penuelas *et al.*, 1994)	WBI	970/900	Water stress
	PRI	$\frac{550-530}{550+530}$	Plant physiology
	NPCI	$\frac{680-430}{680+430}$	Plant physiology

PSSR = Pigment Specific Simple Ratio; PSND = Pigment Specific Normalised Difference; a = Chlorophyll a; b = Chlorophyll b; c = carotenoids; NDVI = Normalised Difference Vegetation Index; SAVI = Soil Adjusted Vegetation Index; WBI = Water Based Index; PRI = Physiological Reflectance Index; NPCI = Normalise Pigments Chlorophyll ratio Index

5.3.2 Statistics

Reflectance data were analysed for any significant differences between treatments at each wavelength, and index data were analysed for any response in the value of the index to treatment. Data analysis was carried out using Microsoft Excel 97. Use of statistical tests involved formulae from (Zar, 1984) entered into an Excel spreadsheet, tested for accuracy using sample data in Minitab. Data were analysed for their suitability for parametric statistical tests. This involved examining the data for a normal distribution around the mean. Given the low number of replicates per wavelength (5 in each treatment), and lack of normal distribution, non-parametric tests were chosen.

The Kruskal-Wallis test and its associated multiple comparison test is a non-parametric version of the one way ANOVA with its multiple comparison test. This was used to test for a significant difference between multiple treatments. The Mann-Whitney U test was used for discerning a significant difference between two samples of data (Zar, 1984). For all statistical tests, significant results are those with a p value of <0.05. Statistical results are shown parenthetically, or where there are many the results for the statistics, tests are shown in graph form with data points indicating which are significant.

5.4 Results: Characterisation

5.4.1 Experiment 1

This experiment was designed to characterise the affect of the lamps heating up on their spectral output. This turned out to be a useful investigation of how the temperature change in the tent affected the performance of the spectroradiometer. In this series the spectroradiometer was kept in the tent with the lights for 30 minutes. The reflectance panel was measured as a target, and should have shown an unchanging reflectance response of 100% as the target was the same as the reference. Instead reflectance increased in the visible and decreased in the infra-red with time (Figure 5.07). However, effects in the infrared were most marked and are likely due to the Pb-based detectors used to measure radiances in these wavelengths and which require electronic cooling to maintain a 'stable' response.

Changes in the spectral output of the lights over this time should have been very minor (pers. comm. D. Emery, NERC EPFS co-ordinator), so other sources of variability were investigated. The GER3700 records temperature data of its sensors, and these showed a strong relationship with reflectance in the near infra red, (Figure 5.08). The temperature change shown is relative to the temperature of the instrument when the reference measurement was taken (at time 0). To allow safe transport of the tent between plots the shroud had to be removed, which would cool the instrument. The temperature of the instrument would therefore be cycling between cooling and heating for a couple of minutes (and so a couple of degrees) as each plot measurement was taken. This initial heating does not have a good relationship with reflectance (Figure 5.09) so it was considered not viable to mathematically correct the reflectance for this heating effect.. In order to minimise the effect of heating on the spectroradiometer and hence to minimise its impact on measured reflectance, the canopy measurements were taken as quickly as possible following the panel reference scan, and only one reflectance measurement of the centre of each plot was made.

5.4.2 Experiment 2

This experiment was designed to characterise the performance of the GER3700 and artificial lighting in the experimental set up by calculating the Signal to Noise Relationship (SNR). The requirement for the study is that the noise should be low enough to allow clear interpretation of the signal. A reference scan of the panel was made, followed by scans of the green nylon scrubbing pads; these scans were taken sequentially immediately after each other. Scans were made for around 1½ minutes, enabling 16 scans to be taken. SNR was calculated by dividing the average reflectance by the standard deviation of reflectance, and the results are presented in Figure 5.10. A high SNR indicates a low variation between replicates. The SNR for the first 6 scans was also calculated, as this better represents the use of the instrument over a single plot (ca. 30 seconds).

These results show a good SNR in the visible and near infra-red, but at wavelengths greater than 1000 nm the SNR is much lower (from approx. 300 to approx. 50). It is still at adequate levels, however, but the magnitude of any response to treatment would have to be greater than required in the visible bands to be discernible. The effect of temperature on the sensor can also be seen here. With the longer period of scanning required to take 16 scans the instrument heats more and reflectance decreases (Fig 5.10), so the noise increases lowering the SNR. With the shorter scanning period used in this study the SNR should be adequate.

5.5 Results: Monoculture canopies

An understanding of the tolerance of the ecotypes used in this study allowed prediction of their responses to metal treatments (Table 5.08). *Fr* Jupiter (NT) was predicted to be stressed by both metal treatments, with a greater spectral effect caused by higher stress in the high zinc treatment. *Fr* Merlin (T) was predicted to either not show stress and so show no spectral response, or be stressed only by the high zinc treatment. Differences between treatments are discussed considering their statistical significance, and if a difference is not statistically significant it is discussed as a qualitative difference. Codes for the treatments which are used throughout this section are given in Table 5.02.

Table 5.08. Predicted results based on knowledge of tolerance for all ecotypes. δ indicates a change in reflectance relative to the control, δδ indicates a greater movement. "-" indicates no difference to the control.

	The treatments reflectance are predicted to respond as following relative to the control	
Prediction for:	Lo Zn	Hi Zn
Fr Jupiter (NT)	δ	δδ
Fr Merlin (T)	-	δ or -

5.5.1 5th September

By the time this dates reflectance measurements were taken the plants had been growing for 131 days, had received 5 fertiliser doses, 150 $\mu g\ g^{-1}$ zinc in the high treatments, and 75 $\mu g\ g^{-1}$ in the low treatments.

Reflectance

All treatments had a fairly similar reflectance, and differences between treatments were hard to discern because of this (Figure 5.11). The Avg/Stdev (and so the similarity in reflectance between replicates of the same treatment) varied across the spectrum, although it was generally fairly low (Figure 5.12). The reflectance results were converted to reflectance difference (Figures 5.13 and 5.14), and differences between treatments were easier to distinguish. Each treatment was compared to the control for that ecotype, a value of 1 indicates that at that wavelength the reflectance of that treatment was 1 percentage reflectance unit higher than the control. Significant differences between treatments are also presented on these figures. *Fr* Jupiter (NT) (Figure 5.13) showed little effect of treatment in the visible region of the spectrum. In the near infra-red reflectance was much higher for JH than JL relative to JC, and in the middle infra-red reflectances for all treatments were once again fairly similar. Statistically significant differences in reflectance were only found in the middle infra-red. *Fr* Merlin (T) (Figure 5.14) showed a much greater response to treatment than *Fr* Jupiter (NT). ML showed slightly higher reflectance in the visible region, with a change in shape of the spectrum (green reflectance was increased relative to blue and red). In the near infra-red ML had increased reflectance. MH showed less of a response to treatment with reflectance being very similar to MC in the visible and near infra-red. There was a significant difference between the low and high zinc treatments in the blue/green and near infra-red. There was no significant difference between the control and any metal treatment at any wavelength.

Vegetation Indices

The statistical results from the ratios used are given in Table 5.09. No ratio showed a significant difference between treatments. Some ratios indicated on Table 5.09 would have shown a significant difference between JC and JH if one of the JC plots, Plot 9, had an index result within the range of the other JC plots. Figure 5.15 shows the results for the (Blackburn, 1998) PSSRa index, typical of those that would be significant but for one "outlier", marked in red. If this "outlier" was within the range of the other values the ratio would be able to distinguish the JC result from the JH. The reflectance results for all JC plots are given in Figure 5.16. All except Plot 9 show similar results across the spectrum, but Plot 9 showed a higher green peak relative to the red trough, and much higher near infra-red reflectance. While it might be argued that Plot 9 could be dismissed as an "outlier", the low number of replicates means that it cannot be certain that it is, and the non-significant index results should stand.

Red Edge

The position of the red edge had no relationship with treatment (*Fr* Jupiter(NT) KW=1.235, P>0.1; *Fr* Merlin(T) KW=1.545, p>0.1), varying from 720-727 nm for all treatments (Figure 5.17). The shape of the first derivative around the red edge did not change between different ecotypes and treatments (Figure 5.18) with all having a dominant long wavelength shoulder.

Summary

- Reflectance showed a significant response to treatment at some wavelengths, not in accordance with predictions based on tolerance.
- No ratios showed a significant response to treatment for either tolerant or non tolerant ecotypes

- REP and the shape of the first derivative showed no response to treatment for either non tolerant or tolerant ecotype.

Discussion

The predicted results bore no relation to the actual results. These spectral measurements were taken with the lowest levels of zinc contamination, 150 $\mu g\ g^{-1}$ added to the high treatment, and 75 $\mu g\ g^{-1}$ added to the low treatment. It is possible that the lack of spectral response can be attributed to these low inputs. The high level of difference between the *Fr* Merlin (T) control and low treatments has an unknown cause, particularly as there is no difference between the control and high treatments. While the experimental set up was designed so that only metal treatment varied between replicates a stochastic variation between plots, such as varience in environmental factors, must cause this difference.

Table 5.09. Vegetation Indices used to find a response to treatment 5th Sep. See Appendix A, Table A5.09 for statistical test results.

Author	Name of Index	Formulae	Result
(Blackburn, 1998)	PSSRa	800/675	N/S τ
	PSSRb	800/650	N/S τ
	reformed PSSRa	800/680	N/S τ
	reformed PSSRb	800/635	N/S τ
	PSSRc	800/500	N/S
	PSNDa	$\frac{800-680}{800+680}$	N/S τ
	PSNDb	$\frac{800-635}{800+635}$	N/S τ
(Carter and Miller, 1994)		695/760	N/S τ
(Malthus *et al.*, 1995)		425/470	N/S
		446/477	N/S τ
		541/836	N/S
		818/538	N/S
		818/713	N/S
(Penuelas *et al.*, 1994)	WBI	970/900	N/S
	PRI	$\frac{550-530}{550+530}$	N/S
	NPCI	$\frac{680-430}{680+430}$	N/S
(Carter and Miller, 1994)		694/420	N/S
		600/760	N/S
		694/760	N/S τ
		VIS/760	N/S τ
(Dawson *et al.*, 1999)		NDVI	N/S τ
		SAVI	N/S τ
This Study	GP/RT	GP/RT	N/S
	GP/990	GP/990	N/S τ
	990/RT	990/RT	N/S τ
	652/605	652/605	N/S

N/S indicates a statistically non-significant difference between treatments of the same ecotype at $p<0.05$. τ indicates that JC would have been significantly different from JH if the index results for Plot 9 were within the range of the other plots.

5.5.2 16th September

By the time this dates reflectance measurements were taken the plants had been growing for 142 days, had received a total of 5 fertiliser doses, 300 $\mu g\ g^{-1}$ zinc in the high treatments, and 150 $\mu g\ g^{-1}$ in the low treatments.

Chlorophyll

The chlorophyll analysis results showed a significant difference between JC and JH for both chlorophylls *a* and *b* (chl a *Fr* Jupiter(NT) KW=7.26, $p<0.05$; chl b *Fr* Jupiter(NT) KW=5.82, $p<0.05$), and no significant difference between *Fr* Merlin (T) treatments (Chl a *Fr* Merlin(T) KW=0.1538, $p>0.10$; Chl b *Fr* Merlin(T) KW=0.038, $p>0.10$; Figure 5.19). Standard deviations around the mean were generally low, but the JL results showed high variances. These results were compared to the predictions based on tolerance, and were used in assessing the reflectance results (Table 5.10). The *Fr* Jupiter (NT) treatments did not comply fully with the prediction based on biology of tolerance as the low zinc treatment showed no difference from the control, although this treatment did have the largest standard deviation. The *Fr* Merlin (T) results were as expected with no change in chlorophyll concentration between treatments.

Table 5.10. Predicted results based on knowledge of tolerance and chlorophyll results for all ecotypes 16/9. δ indicates a change in reflectance relative to the control, δδ indicates a greater movement. "-" indicates no difference to the control. Arrows indicate the direction of significant differences.

	The treatments reflectance are predicted to respond as following relative to the control			
	JL	JL	ML	MH
Prediction from Tolerance	δ	δδ	-	δ/-
Chlorophyll results	-	$\downarrow$	-	-

Reflectance

The two ecotype's reflectances were distinct across much of the spectrum with *Fr* Merlin's (T) reflectance being greater than *Fr* Jupiter's (NT) (Figure 5.20). Within ecotypes there was no significant difference in the visible or near infra-red, and results are discussed here as being non-significant differences. The Avg/StDev for all treatments was fairly low, indicating a lot of variation in reflectance within treatments (Figure 5.21). Considering *Fr* Jupiter (NT) treatments, from the start of the visible to the start of the green peak there was no discernible change in the pattern; at each waveband the treatments were close together and they were parallel across wavebands (Figure 5.22). At the green peak (around 550 nm) JC showed a slightly higher reflectance, with JL and JH being very similar. At the red trough however, JC and JL's reflectances were very similar, but JH's reflectance was higher. JH effectively showed a flattened response between the green and red relative to the other *Fr* Jupiter (NT) treatments. At longer wavelengths the reflectances resumed their previous pattern, with no obvious differences with treatment. There was no equivalent change in the pattern of the *Fr* Merlin (T) treatments in the visible region.

The reflective difference graphs (*Fr* Jupiter (NT), Figure 5.23; *Fr* Merlin (T), Figure 5.24), revealed the differences between treatments within cultivars more clearly than the reflectance graphs, although a little more abstractly. A relationship between reflectance and treatment consistent with the predicted relationship can be seen for *Fr* Jupiter (NT) between 500 - 730 nm (Figure 5.23, i.e. the change in reflectance is larger in the high zinc treatment than in the low zinc treatment). Figure 5.23 also seemed to indicate that an index using reflectance at ca. 705 nm would highlight differences between treatments as this is the region of greatest average difference between treatments. However, the Kruskal-Wallis results (Figure 5.25) showed that this wavelength had a weak relationship with treatment ($n=15$, $H=1.47$, $p>0.2$). Wavelengths such as 653 had H values of 4.94 ($p>0.05$), and the red trough (ca. 670 nm) had H values of 3.98 ($p>0.1$). The greatest and only significant difference was at ca. 2480 nm, and this was considered to be unrelated to canopy biochemistry and more related to instrument performance. Given the obvious change in reflectance in response to treatment (Fig. 5.22, 5.23), and favourable KW statistical tests (Fig. 5.25) the red region was included in the development of statistical tests in this study (detailed in the next section, Vegetation Indices).

The *Fr* Merlin (T) reflectance difference graph (Figure 5.24) showed greater differences between treatments than *Fr* Jupiter (NT), the low zinc treatment had consistently higher reflectance than the high treatment, which in turn was consistently brighter than the control. The only significant difference between treatments was once again at around 2480 nm.

Vegetation Indices

Kruskal-Wallis statistics were carried out on the vegetation indices used in this study, with significant results being those with a p value < 0.05 (Table 5.11). None of the indices taken from the literature showed a significant response to treatment. Unlike the results for 9/5 none came close to differentiating between treatments.

Table 5.11. Vegetation Indices used to find a response to treatment 16/9. See Appendix A, Table A5.11 for statistical test results.

Author	Name of Index	Formulae	Result
(Blackburn, 1998)	PSSRa	800/675	N/S
	PSSRb	800/650	N/S
	reformed PSSRa	800/680	N/S
	reformed PSSRb	800/635	N/S
	PSSRc	800/500	N/S
	PSNDa	$\frac{800-680}{800+680}$	N/S
	PSNDb	$\frac{800-635}{800+635}$	N/S
(Carter and Miller, 1994)		695/760	N/S
(Malthus *et al.*, 1995)		425/470	N/S
		446/477	N/S
		541/836	N/S
		818/538	N/S
		818/713	N/S
(Penuelas *et al.*, 1994)	WBI	970/900	N/S
	PRI	$\frac{550-530}{550+530}$	N/S
	NPCI	$\frac{680-430}{680+430}$	N/S
(Carter and Miller, 1994)		694/420	N/S
		600/760	N/S
		694/760	N/S
		VIS/760	N/S
(Dawson *et al.*, 1999)		NDVI	N/S
		SAVI	N/S
This Study	GP/RT	GP/RT	**Sig.** JC : JH
	GP/990	GP/990	N/S
	990/RT	990/RT	N/S
	652/605	652/605	**Sig.** JC : JH

N/S indicates a statistically non-significant difference between treatments of the same ecotype at $p<0.05$. Sig. indicates a statistically significant difference between treatments at $p<0.05$, with letter codes indicating which treatments are different.

Indices were also developed as part of this study. Kruskall-Wallis (KW) results (Figure 5.25) showed that for *Fr* Jupiter (NT) the wavebands best suited for differentiating between treatments lay at 652 nm and 2480 nm. While the reflectance results alone were not significantly different between treatments at 652, this waveband was investigated for being a component of an index. Wavebands near 2480 were not analysed as their reflectance was considered to be anomalous. R_{652} was combined in an index with all other wavebands, and the results tested for a statistically significant difference between treatments (Figure 5.26). The best other wavebands to use were those around 605 nm, KW 9.14, $p<0.005$. This waveband on its own showed no response to treatment (KW -0.2, $p>0.5$; Fig. 5.25). Another of the indices developed as part of this study, the Green Peak / Red Trough index (GP/RT), also showed a significant response to treatment (*Fr* Jupiter(NT) KW=9.14, $p<0.05$). This index showed a significant difference between the JC and JH treatments (*Fr* Jupiter(NT) KW=9.14, $p<0.05$; Figure 5.27). Both GP and RT reflectances were also incorporated into indices involving near infra red reflectance(GP/990 and 990/RT). These indices were developed using this date's data and carried onto the results from later dates. Both of these indices characterise the flattening of the spectral response in the JH treatment in the red and green regions of the spectrum. This flattening is probably in response to lower chlorophyll concentrations resulting in lower absorption of light in the visible spectrum. As chlorophyll has a greater effect on absorbance in the red, a decrease in chlorophyll concentration will increase red reflectance. The location of the wavebands used in these indices is shown in Figure 5.28.

Red Edge

The red edge position showed no statistically significant relationship with treatment, covering a range from 705 to 725 nm (Figure 5.29). Kruskal Wallis tests within the cultivars revealed no significant difference between treatments (*Fr* Jupiter (NT)

$H=4.265$, $p>0.10$; *Fr* Merlin (T) $H=1.5$, $p>0.20$). Except for JH and MC the range of values was low. The average treatment first derivative results showed a subtle change in shape in the red edge region between treatments (Figure 5.30). All the *Fr* Merlin (T) treatments had an obvious long shoulder peak, as did JC. JL and JH had a flattened peak, indicating a decreasing dominance by the long shoulder. The range in REP values for JH came from some replicates having short wavelength dominant peaks, and others having long wavelength dominant peaks. The first derivative also showed the flattening in the reflectance of the JH treatment in the red trough region as first derivative values in that region were closer to zero (Fig. 5.30).

Summary

- Chlorophyll concentration decreased in the non-tolerant plants with metal application, but not in the tolerant plants.
- Reflectance showed no significant response to treatment.
- *Fr* Jupiter (NT) high zinc showed less of a red trough than other treatments (non-significant).
- Ratios from the literature showed no significant difference between treatments
- Ratios developed in this study showed a significant response to treatment for *Fr* Jupiter (NT).
- Red edge position was not significantly different between cultivars/treatments.
- The shape of the first derivative curve around the REP did change with treatment (JC - long shoulder dominant, JH - short shoulder dominant).

Discussion

This data set had higher doses of metals applied, being 300 $\mu g\ g^{-1}$ on the high treatments, and 150 $\mu g\ g^{-1}$ on the low treatments. There was a significant decrease in chlorophyll levels between *Fr* Jupiter (NT) control and high treatments, indicating stress, and no change in *Fr* Merlin (T). The *Fr* Jupiter (NT) high zinc treatment showed non-significant changes in reflectance around the red absorption feature with metal treatment. This region is one of the main absorption features of chlorophyll, and for reflectance to change in this region there has to be a large reduction in chlorophyll (Verhoef, 2000). However there was no concomitant change in reflectance in other regions in the visible, and indices from the literature designed to detect either changes in pigment concentration or stress were unsuccessful. Indices developed in this study to highlight changes in the green-red region (GP/RT; 652/605) were successful in differentiating the *Fr* Jupiter (NT) control from the high treatment. While the REP did move to shorter wavelengths in some JH replicates, there was no significant change in REP. Given the qualitative difference in chlorophyll concentration and reflectance in the red trough, a clear difference in red edge position would be expected, as this is recommended as a more sensitive and earlier indication of stress (and chlorophyll concentration) than any other method (Vogelmann *et al.*, 1993; Curran *et al.*, 1991). This did not happen, although the shape of the first derivative around the red edge position appears to be starting to show the two-shoulder pattern intimated in the literature (Filella and Penuelas, 1994).

Fr Merlin, the tolerant ecotype, shows no qualitative or quantitative response to the higher metal concentrations.

5.5.3 15th October

By the time this dates reflectance measurements were taken the plants had been growing for 161 days, had received a total of 5 fertiliser doses, 450 $\mu g\ g^{-1}$ zinc in the high treatments, and 225 $\mu g\ g^{-1}$ in the low treatments.

Chlorophyll

The chlorophyll results showed no significant difference between treatments (Chl a: *Fr* Jupiter (NT) KW=3.11, p>0.1; *Fr* Merlin (T) KW=2, p>0.1; Chl b: *Fr* Jupiter (NT) KW=4.88, p>0.1; *Fr* Merlin (T) KW=2.34, p>0.1; Figure 5.31). These results were compared to the predictions based on tolerance, and were used in assessing the reflectance results (Table 5.12). The *Fr* Jupiter (NT) chlorophyll results did not comply with the prediction based on tolerance as there was no change, while the *Fr* Merlin (T) chlorophyll results were not predicted to change, and did not change.

Table 5.12. Predicted results based on knowledge of tolerance and chlorophyll results for all ecotypes, 15/10. δ indicates a change in reflectance relative to the control, δδ indicates a greater movement. "-" indicates no difference to the control.

	The treatments reflectance are predicted to respond as following relative to the control			
	JL	JL	ML	MH
Prediction from Tolerance	δ	δδ	-	δ/-
Chlorophyll results	-	-	-	-

Reflectance

There were no significant differences between treatment reflectances across the entire spectrum, and all differences discussed are qualitative. The mean treatment reflectance results showed some response to cultivar with the *Fr* Merlin (T) treatments being brighter than the *Fr* Jupiter (NT) treatments below 1400 nm, except in the red trough region (Figure 5.32). The Avg/StDev was generally low, indicating a considerable variation in reflectance between replicates of the same treatment, (Figure 5.33). The only qualitative treatment reflectance response for *Fr* Jupiter (NT) was between the green reflectance peak and the red trough (Figure 5.34). In the blue region the reflectance of all treatments was fairly similar. At the green peak the JC treatment was higher than either JL or JH. At the red trough the JC and JL treatments were much closer together, while the JH treatment was higher than both (Fig. 5.34). *Fr* Merlin (T) showed a similar, although much smaller response, with the MH and MC treatments being identical in the green, but MH was slightly higher than MC in the red (Fig. 5.34).

Reflectance difference graphs for *Fr* Jupiter (NT) highlighted the green peak - red trough differences, and also revealed differences between treatments in the near infra red (Figure 5.35). The rest of the spectrum showed little response to treatment. There was little difference between treatments for *Fr* Merlin (T) (Figure 5.36).

Vegetation Indices

None of the indices taken from the literature distinguished between treatments (Table 5.13). However, indices (GP/RT, 652/605) developed during this study did differentiate between the *Fr* Jupiter (NT) control and high zinc treatments (GP/RT in Figure 5.37).

Red Edge

The position of the red edge was in significantly shorter wavelengths for the high *Fr* Jupiter (NT) treatment than the other *Fr* Jupiter (NT) treatments (Kruskal-Wallis, $H=7.595$, $p<0.05$, Figure 5.38). *Fr* Merlin (T) showed no significant response of REP to treatment (Kruskal-Wallis, $H=0.56$, $p>0.05$) (Fig. 5.38). The spread of REP in most treatments was quite large, 16 nm for JC, 21 nm for ML, and just 4 nm for JH. In the *Fr* Jupiter (NT) treatments the red edge peak region of the first derivative was flat except for the high treatment where the short wavelength shoulder is dominant (Figure 5.39). For *Fr* Merlin (T) the average treatment first derivative results were very similar for all treatments, with the long wave shoulder dominant around the red edge position (Fig 5.39). Statistical tests on the first derivative results did not yield a significant response of treatment within cultivars for *Fr* Jupiter (NT) or *Fr* Merlin (T).

Table 5.13. Vegetation Indices used to find a response to treatment 10/15. See Appendix A, Table A5.13 for statistical test results.

Author	Name of Index	Formulae	Result
(Blackburn, 1998)	PSSRa	800/675	N/S
	PSSRb	800/650	N/S
	reformed PSSRa	800/680	N/S
	reformed PSSRb	800/635	N/S
	PSSRc	800/500	N/S
	PSNDa	$\frac{800-680}{800+680}$	N/S
	PSNDb	$\frac{800-635}{800+635}$	N/S
(Carter and Miller, 1994)		695/760	N/S
(Malthus *et al.*, 1995)		425/470	N/S
		446/477	N/S
		541/836	N/S
		818/538	N/S
		818/713	N/S
(Penuelas *et al.*, 1994)	WBI	970/900	N/S
	PRI	$\frac{550-530}{550+530}$	N/S
	NPCI	$\frac{680-430}{680+430}$	N/S
(Carter and Miller, 1994)		694/420	N/S
		600/760	N/S
		694/760	N/S
		VIS/760	N/S
(Dawson *et al.*, 1999)		NDVI	N/S
		SAVI	N/S
This Study	GP/RT	GP/RT	**Sig**. JC : JH
	GP/990	GP/990	N/S
	990/RT	990/RT	N/S
	652/605	652/605	**Sig.** JC : JH

N/S indicates a statistically non-significant difference between treatments of the same ecotype at $p<0.05$.
Sig. indicates a statistically significant difference between treatments at $p<0.05$, with letter codes indicating which treatments are different.

Summary

- There was no significant difference in chlorophyll concentrations or reflectances between treatments.
- The *Fr* Jupiter (NT) high and low treatments showed an increased reflectance in the red trough relative to control.
- The *Fr* Merlin (T) high treatment showed a slight increase in reflectance in the red trough relative to the control.
- Indices from the literature were unsuccessful in differentiating treatments.
- Some indices developed during this study were successful in differentiating JC and JH
- Red edge position showed a significant effect of treatment for *Fr* Jupiter (NT), moving to shorter wavelengths with the high zinc treatment.
- *Fr* Merlin (T) showed no change in red edge position with treatment.

Discussion

This data set has the longest and highest zinc exposure, 450 $\mu g\ g^{-1}$ for the high treatment and 225 $\mu g\ g^{-1}$ for the low treatment, but showed no significant difference in chlorophyll concentration. This data set also showed the least quantitative (statistically significant) differences between treatment reflectances, as would be expected from the chlorophyll results but not the metal exposure history. The reflectance of high zinc treatments of both ecotypes did show a qualitative (non-significant) response to treatment around the red trough, although the response of *Fr* Merlin (T) was very slight. Vegetation indices gathered from the literature were once again unable to distinguish between treatments. Those developed in this study using the red trough region did show a significant effect of treatment in the *Fr* Jupiter (NT) ecotype. There was a significant decrease in red edge position with zinc application for *Fr* Jupiter (NT), with the first derivative curves showing a dominant short wavelength shoulder.

Fr Merlin (T) once again showed no quantitative response to treatment, and only a very minor qualitative response (in the red reflectance region).

5.5.4 Changes in reflectance over time

This section analyses the changes in results over the three measurement periods. How the results relative to other treatments on the same date change over time (e.g. is there a REP response of JH relative to JC on all dates?) is analysed, as is how the results of the same treatment change relative to that treatment's results on different dates (e.g. is there a change in REP of JH over time?). A summary of all the statistically significant results from each date is presented in Table 5.14.

Table 5.14. Predicted and actual results for all ecotypes in monoculture over time. δ indicates a change in reflectance relative to the control, $\delta\delta$ indicates a greater movement. "-" indicates no difference to the control. Arrows indicate a significant direction of movement, and where an index significantly discriminated between treatments it is stated.

	The treatments reflectance are predicted to respond/did respond as following relative to the control for each ecotype			
Prediction from:	JL	JH	ML	MH
Tolerance	δ	$\delta\delta$	-	δ/-
Result from:				
5 September - Reflectance	$\lambda_{some}\uparrow R$	-	-	-
- Indices	-	-	-	-
- REP	-	-	-	-
16 September - Chlorophyll	-	$\downarrow$	-	-
- Reflectance	-	-	-	-
- Indices	-	GP/RT, 652/605	-	-
- REP	-	-	-	-
15 October - Chlorophyll	-	-	-	-
- Reflectance	-	-	-	-
- Indices	-	GP/RT	-	-
- REP	-	$\downarrow$	-	-

$\lambda_{some}\uparrow R$ = at some wavelengths there was a significant increase in reflectance.

Chlorophyll

Chlorophyll concentration was only significantly different between treatments in the second data set (Table 5.14). No treatments showed a significant change in chlorophyll concentration over time, and there was generally a high variation in treatments chlorophyll concentrations (MWU; JC, U=15, $p>0.05$; JL, U=13, $p>0.05$; JH, U=8, $p>0.05$; MC, U=12, $p>0.05$; ML, U=9, $p>0.05$; MH, U=11, $p>0.05$; Figure 5.40).

Reflectance

There was little significant difference in reflectance between treatments over the study period (Table 5.14). Only on the first date, with the least exposure to metal was there a significant difference between treatments and only in near infra-red wavelengths. However, considering the change in reflectance of the same treatment over the study period there were significant differences (JC Fig 5.41, JL Fig 5.42, JH Fig 5.43, MC Fig 5.44, MM Fig 5.45, MH Fig 5.46). All treatments show a significant increase in reflectance at most wavelengths with time. The *Fr* Merlin (T) high treatment shows a significant difference in reflectance with time across the near infra red (Fig. 5.46), other treatments do not.

The change in green and red reflectance in response to treatment over time can be seen in the *Fr* Jupiter (NT) treatments (Figures 5.41 through 5.43). The control treatment maintains a steep green slope and definite red trough over all dates (Fig. 5.41). The low zinc treatment shows a slight flattening of the red trough (Fig. 5.42), while the high treatment shows a marked flattening of this region with time, especially between the second and final measurements (Fig 5.43). The *Fr* Merlin (T) treatments show no obvious change in spectral shape over time (Figs 5.44 through 5.46).

Vegetation Indices

Indices gathered from the literature to measure stress and/or pigments were unsuccessful in differentiating between treatments on single measurement dates. However, indices developed during this study (GP/RT and 652/605) were successful in differentiating between JC and JH treatments on the 16/9 and 15/10 (Table 5.14). Comparing index results for the same treatment over time, most vegetation indices did show a response (Table 5.15) e.g. for the JH treatment, all indices were significantly different if compared over time except for GP/990. The results for the GP/RT index for each treatment over time are given in Figure 5.47. The lines are given for clarity, not to infer where actual index values could lie. JL and JH treatments showed a consistent decrease in index value over time. The index values of the JC and all *Fr* Merlin (T) treatments increased between the first and second measurements, and decreased in the third measurement. This indicated that a decrease in the GP/RT index was a general phenological feature of this species, and began earlier in stressed canopies.

Red Edge Position

REP was only successful in differentiating between treatments on the final measurement dates (Table 5.14). The change in REP for each treatment over time showed JC having no significant response over time, while the other treatments all show a significant decrease in REP over time (KW; JC=3.85, $P>0.05$; JL=8.73, $P<0.05$; JH=10.59, $P<0.05$; MC=8.54, $P<0.05$; ML=6.36, $P<0.05$; MH=8.65, $P<0.05$; Figure 5.48). All treatments start with their average REP at around 723 nm. At the time of the final measurement JH's average REP is at 702.5 nm, other treatment REP varies from 712 to 720 nm.

Table 5.15. Vegetation index results for each monoculture treatment over time. See Appendix A, Table A5.15 for statistical test results.

Author	Formulae	JC	JL	JH	MC	ML	MH
(Blackburn, 1998)	800/675	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	800/650	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	800/680	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	800/635	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	800/500	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	$\frac{800-680}{800+680}$	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	$\frac{800-635}{800+635}$	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
(Carter and Miller, 1994)	695/760	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
(Malthus *et al.*, 1995)	425/470	Sig.	Sig.	Sig.	Sig.	N/S	N/S
	446/477	Sig.	Sig.	Sig.	Sig.	N/S	N/S
	541/836	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	818/538	Sig.	Sig.	Sig.	Sig.	N/S	N/S
	818/713	Sig.	Sig.	Sig.	Sig.	N/S	N/S
(Penuelas *et al.*, 1994)	970/900	N/S	Sig.	Sig.	Sig.	N/S	N/S
	$\frac{550-530}{550+530}$	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	$\frac{680-430}{680+430}$	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
(Carter and Miller, 1994)	694/420	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	600/760	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	694/760	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	VIS/760	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
(Dawson *et al.*, 1999)	NDVI	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	SAVI	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
This Study	GP/RT	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	GP/990	N/S	Sig.	N/S	Sig.	N/S	Sig.
	552/605	N/S	Sig.	Sig.	N/S	Sig	Sig
	990/RT	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.

N/S indicates a statistically non-significant difference between treatments at $p<0.05$.

The first derivative curves in the region of the red edge showed the range of response to treatment in the different ecotypes. The *Fr* Jupiter (NT) control treatments showed a dominant long wavelength shoulder at first, and in the second data set, but by the third data collection there was a flattening of the curve, with the short wavelength shoulder becoming more dominant, although the peak was still at the longer wavelengths (Figure 5.49). The JL treatment showed a much more marked flattening in the red edge region with time (Figure 5.50). The *Fr* Jupiter (NT) high treatments also started with a long wavelength dominant shoulder, the second data set had the curve flattening (shoulders equally important) and the final data set had the short wavelength shoulder obviously dominant (Figure 5.51). The *Fr* Merlin (T) treatments all showed similar results to each other, with a small degree of flattening in red edge region in the last data set, with prior dates having a long wavelength dominant peak (MC Fig. 5.52; ML Fig. 5.53; MH Fig. 5.54).

Summary

- Chlorophyll concentrations showed little response to treatment over time.
- Reflectance showed a significant increase with time across much of the spectrum for all treatments.
- Most indices showed a change in values with time for all treatments.
- The red edge position of all treatments moved to shorter wavelengths with time.
- First derivative curves showed an increased dominance of the short wavelength shoulder with time.

Discussion

Despite there being no significant change in chlorophyll concentration over time, all treatment reflectance measures (reflectances, all indices, red edge positions) showed a significant change compatible with a change in chlorophyll concentration or stress. That the control and tolerant ecotypes showed the same pattern as the other treatments indicates this response was due to factors other than metal stress. *Fr*

Jupiter (NT) high zinc treatment responses were greater over time (e.g. REP for all treatments moved to shorter wavelengths, REP moved to shorter wavelengths earlier in the season, and the magnitude of movement was greater by the end of the season). The most likely reason for all treatments responding is the changing of the plants physiological properties over time with phenology (i.e. changes over the growth season, senescence). This should have been identifiable in the chlorophyll results; that it wasn't indicates either the analysis technique was deficient or other factors contribute to reflectance. Leaf biochemistry, and leaf and canopy structural changes could all contribute towards these differences.

5.6 Results: Mixtures

5.6.1 October 22

By the time this dates reflectance measurements were taken the plants had been growing for 118 days, had received a total of 2 fertiliser doses, 300 $\mu g\ g^{-1}$ zinc in the high treatments, and 150 $\mu g\ g^{-1}$ in the low treatments.

<u>Chlorophyll</u>

There was no significant difference in chlorophyll concentration between treatments (KW; Chl a KW = 1.67, $p>0.05$; Chl b KW = 4.88, $p>0.05$; Figure 5.55). No prediction of the results based on tolerance is thus possible; with the ecotypes being mixed in the plot the relative contribution of each to reflectance is unknown. However, a prediction based on chlorophyll results of no difference between the reflectance responses of treatments can be made (Table 5.16).

Table 5.16. Prediction of remote sensing responses based on knowledge of the biology of tolerance and chlorophyll concentration results for mixed plots 22/10. "N/P" means Not Possible, and "-" denotes that no change is predicted.

	The treatments reflectance are predicted to respond as following relative to the control for each ecotype	
Prediction from:	Low	High
Tolerance	N/P	N/P
Chlorophyll	-	-

<u>Reflectance</u>

The mean treatment reflectance showed a significant response to treatment in the visible and near infra-red wavelength regions (Figure 5.56). All treatments had a fairly flat response between the green and infra-red regions with no red trough. The Avg/StDev value for treatments was fairly variable across the spectrum, but was generally low indicating a lot of variation between replicates (Figure 5.57). The reflective difference graph shows that across much of the spectrum the low zinc treatment had a lower reflectance than the control, and the high treatment's reflectance was lower than the low zinc (Figure 5.58). This was a dose response relationship, with higher metal doses decreasing reflectance more than low doses.

Vegetation Indices

The vegetation indices showed a lot more success in differentiating between treatments than in the monoculture experiments (Table 5.17). Two indices formulated to detect changes in chlorophyll b concentrations were successful (Blackburn's reformed PSSRb and PSNDb), along with some general stress detection indices (e.g. Carter's 695/760 and Vis/760, and this study's GP/RT). Of these only the GP/RT was successful on monocultures. Other indices that were successful in this instance, but unsuccessful in previous monoculture data sets were the NDVI and SAVI indices.

Red Edge Position

There was a significant difference between the REP of the control and low zinc treatment (MWU; $U = 16$, $P<0.05$), with the high zinc treatment showing no significant difference to the others (MWU; Control vs High, $U=14$, $p>0.05$; Low vs. High, $U=13$, $p>0.05$; Figure 5.59). The low zinc's REP (701-703 nm) was at significantly shorter wavelengths than the control (706-716 nm). The first derivative results showed a flat region around the red edge for the control, a distinctive short wavelength peak for the low treatment and a less obvious short wavelength peak for the high zinc treatment (Figure 5.60).

Table 5.17. Vegetation Indices used to find a response to treatment 22/10. See Appendix A, Table A5.17 for statistical test results.

Author	Name of Index	Formulae	Result
(Blackburn, 1998)	PSSRa	800/675	N/S
	PSSRb	800/650	Sig.
	reformed PSSRa	800/680	N/S
	reformed PSSRb	800/635	N/S
	PSSRc	800/500	N/S
	PSNDa	$\frac{800-680}{800+680}$	N/S
	PSNDb	$\frac{800-635}{800+635}$	Sig.
(Carter and Miller, 1994)		695/760	Sig.
(Malthus *et al.*, 1995)		425/470	N/S
		446/477	N/S
		541/836	N/S
		818/538	N/S
		818/713	N/S
(Penuelas *et al.*, 1994)	WBI	970/900	N/S
	PRI	$\frac{550-530}{550+530}$	N/S
	NPCI	$\frac{680-430}{680+430}$	N/S
(Carter and Miller, 1994)		694/420	N/S
		600/760	N/S
		694/760	N/S
		VIS/760	Sig.
(Dawson *et al.*, 1999)		NDVI	Sig.
		SAVI	Sig.
This Study	GP/RT	GP/RT	Sig.
	GP/990	GP/990	N/S
	652/605	652/605	Sig.
	990/RT	990/RT	N/S

N/S indicates a statistically non-significant difference between treatments at $p<0.05$. In all cases Sig. indicates a statistically significant difference between the index result of the control and high zinc treatment.

Summary

- There was no significant difference in chlorophyll concentration.
- There was a significant difference between treatment reflectances in the visible and near infra-red.
- Several indices were successful at differentiating between treatments (control and high zinc).
- There was a significant response of REP between the control and low zinc treatments.

Discussion

Although there was no significant difference in chlorophyll concentrations between treatments there were reflectance responses. Given the absence of reflectance responses detected in the monoculture experiments for *Fr* Merlin (T) and few responses by *Fr* Jupiter (NT) this was unexpected from a mixture of the two ecotypes. Reflectance decreased significantly relative to the control in the high zinc treatment in the green and near infra-red wavebands. A number of indices were successful at differentiating between the control and high treatments, including two formulated to distinguish vegetation amount (NDVI and SAVI). More indices were successful for this data set than any other (monoculture or mixture). REP differentiated the control from the low zinc treatment, but not the high zinc treatment, a surprising result given that the low treatment was statistically indistinct from the control in terms of reflectance or vegetation indices. Interestingly, if the REP responses from the monoculture experiments for the same treatment in both ecotypes (Oct. 15th monoculture data) are combined to simulate a mixed canopy, the results are dissimilar from the results from a physically mixed canopy (Oct 22nd mixed data; Figure 5.61). If the monoculture REP's for both cultivars are combined for the control and high treatments, the results would not be too dissimilar to those for the mixed canopy. However, the low treatments REP's for the mixed canopy are at shorter wavelengths than would be predicted by simply mixing the results from the monoculture experiments. The cause of this result is not clear.

5.6.2 November 16th

By the time this dates reflectance measurements were taken the plants had been growing for 150 days, had received a total of 2 fertiliser doses, 300 $\mu g\ g^{-1}$ zinc in the high treatments, and 150 $\mu g\ g^{-1}$ in the low treatments.

Chlorophyll

Although qualitatively, average chlorophyll concentration shows a decrease with increasing zinc dose, there was no significant difference in concentration between the treatments (Figure 5.62).
A prediction of the results based on tolerance is not possible, but a prediction based on chlorophyll results of no difference between the reflectance responses of treatments can be made (Table 5.18).

Table 5.18. Prediction of remote sensing responses based on knowledge of the biology of tolerance and chlorophyll concentration results 16/11. "N/P" means Not Possible, and "-" denotes that no change is predicted.

	The treatments reflectance are predicted to respond as following relative to the control for each ecotype	
Prediction from:	Low	High
Tolerance	N/P	N/P
Chlorophyll	-	-

Reflectance

There were significant differences between the control and high zinc treatment reflectances in the visible and near infra-red spectral regions (Figure 5.63). The Avg/StDev was low across the spectrum, so variation within replicates was fairly high (Figure 5.64). The high zinc treatment reflectance was lower than the low zinc treatment reflectance, which in turn is lower than the control, indicating a dose response relationship (Figure 5.65). All treatments exhibit a fairly flat response in the green-red region with no obvious red trough.

Vegetation Indices

No indices showed a significant response to treatment (Table 5.19).

Red Edge Position

There was no significant difference between treatments REP ($KW=1.19$, $p>0.05$; Figure 5.66). All treatments showed a REP at similar wavelengths, at around 702 nm, with all having a dominant short wavelength peak of first derivative (Figure 5.67).

Summary

- There was no significant response of chlorophyll concentration to treatment.
- Reflectance was lower in the high treatment in the green and near infra-red wavebands.
- No indices differentiated between treatments.
- REP showed no response to treatment.

Table 5.19. Vegetation Indices used to find a response to treatment 16/11. See Appendix A, Table A5.19 for statistical test results.

Author	Name of Index	Formulae	Result
(Blackburn, 1998)	PSSRa	800/675	N/S
	PSSRb	800/650	N/S
	reformed PSSRa	800/680	N/S
	reformed PSSRb	800/635	N/S
	PSSRc	800/500	N/S
	PSNDa	$\frac{800-680}{800+680}$	N/S
	PSNDb	$\frac{800-635}{800+635}$	N/S
(Carter and Miller, 1994)		695/760	N/S
(Malthus *et al.*, 1995)		425/470	N/S
		446/477	N/S
		541/836	N/S
		818/538	N/S
		818/713	N/S
(Penuelas *et al.*, 1994)	WBI	970/900	N/S
	PRI	$\frac{550-530}{550+530}$	N/S
	NPCI	$\frac{680-430}{680+430}$	N/S
(Carter and Miller, 1994)		694/420	N/S
		600/760	N/S
		694/760	N/S
		VIS/760	N/S
(Dawson *et al.*, 1999)		NDVI	N/S
		SAVI	N/S
This Study	GP/RT	GP/RT	N/S
	652/605	652/605	N/S
	GP/990	GP/990	N/S
	990/RT	990/RT	N/S

N/S indicates a statistically non-significant difference between treatments at $p<0.05$.

Discussion

This data set with the longest metal exposure showed the least response to metal. There was no chlorophyll difference between treatments, limited reflectance response and no index or REP response. This lack of response could be because the measurements were taken so late in the season, and all plants were in late stages of senescence. There is no mention in the literature of a difference in the extent of senescence between stressed and unstressed plants, so they are likely to senesce to the same extent and show the same end of season physiology, and so the same end of season reflectance.

5.6.2 Changes over time

This section analyses the change in results in the mixed canopies over time. How the results relative to other treatments on the same date change over time (e.g. is there a reflectance response of the low zinc relative to the control on all dates?) is analysed, as is how the results of the same treatment change relative to that treatments results on different dates (e.g. is there a change in REP of the high zinc treatment over time?). A summary of all the statistically significant results from each date are presented in Table 5.20.

Table 5.20. Predicted results based on knowledge of tolerance, and chlorophyll along with remote sensing results for all ecotypes over time for the mixture experiment. "N/P" indicates that it is not possible to predict based on this information. "-" indicates there is no difference relative to the control. Arrows indicate a significant direction of movement, and where an index significantly discriminated between treatments its identity is stated.

	The treatments reflectance are predicted to respond/did respond as following relative to the control for each ecotype	
	Low Zinc	High Zinc
Prediction from:		
Tolerance	N/P	N/P
Result from:		
22 October - Chlorophyll	-	-
- Reflectance	-	$\lambda_{some}\downarrow R$
- Indices	-	PSSRb; PSNDb; NDVI; SAVI; GP/RT
- REP	$\downarrow$	-
16 November - Chlorophyll	-	-
- Reflectance	-	$\lambda_{some}\downarrow R$
- Indices	-	-
- REP	-	-

$\lambda_{some}\downarrow R$ = at some wavelengths there was a significant decrease in reflectance.

Chlorophyll

There was no significant change in chlorophyll concentration between treatments on any date (Table 5.20). There was also no statistically significant change in chlorophyll concentration for any one treatment over time (Figure 5.68).

Reflectance

On both dates there was a significant difference between the control and high zinc treatments at similar wavelengths, near the green peak, and in the near infra-red wavelength region (Figure 5.69). On both dates the high treatment reflectance was significantly lower than the control treatments. The control treatment reflectance increased significantly over time in the red trough region and at some wavebands in the near infra-red (Figure 5.70). Both the low (Figure 5.71) and high treatments (Figure 5.72) showed a similar response over time.

Vegetation Indices

On the first measurement date five indices taken from the literature were able to differentiate between treatments, while on the second date no indices were able to distinguish one treatment from another (Table 5.20). When comparing the index results for the same treatment over time most indices did show significant differences within treatments between dates (Table 5.21).

Red Edge Position

REP for all treatments moved to very similar short wavelengths with time (Figure 5.73). However, the control treatment was the only one to move to a significantly different wavelength (Mann-Whitney $U = 16$, $p<0.05$), and shows the greatest shortening of REP.

Summary

- There was no change in chlorophyll concentrations over time.
- Reflectance generally increased over time.
- Most vegetation indices values changed over time within treatments.
- The REP moved to shorter wavelengths over time.

Discussion

There were no significant changes in chlorophyll concentration over time, although reflectance responses in all treatments (reflectances, indices, red edge positions) showed a significant change in one or more treatments. The most likely reason for all treatments responding in a similar manner is that the plant physiological properties are changing over time with phenology (i.e. changes over the growth season, senescence). Changes in the canopy properties other than chlorophyll content such as leaf biochemistry, and leaf and canopy structural changes could all contribute towards these differences.

Table 5.21. Response of Vegetation Indices for the same mixture treatments over time. See Appendix A, Table A5.21 for statistical test results.

Author	Formulae	Control	Low Zinc	High Zinc
(Blackburn, 1998)	800/675	Sig.	Sig.	Sig.
	800/650	Sig.	Sig.	Sig.
	800/680	Sig.	Sig.	Sig.
	800/635	Sig.	Sig.	Sig.
	800/500	Sig.	Sig.	N/S
	$\frac{800-680}{800+680}$	Sig.	Sig.	Sig.
	$\frac{800-635}{800+635}$	Sig.	Sig.	Sig.
(Carter and Miller, 1994)	695/760	Sig.	Sig.	N/S
(Malthus *et al.*, 1995)	425/470	Sig.	Sig.	N/S
	446/477	Sig.	Sig.	N/S
	541/836	Sig.	Sig.	Sig.
	818/538	Sig.	Sig.	Sig.
	818/713	Sig.	Sig.	Sig.
(Penuelas *et al.*, 1994)	970/900	N/S	Sig.	Sig.
	$\frac{550-530}{550+530}$	Sig.	Sig.	Sig.
	$\frac{680-430}{680+430}$	Sig.	Sig.	Sig.
(Carter and Miller, 1994)	694/420	Sig.	Sig.	Sig.
	600/760	Sig.	Sig.	Sig.
	694/760	Sig.	Sig.	N/S
	VIS/760	Sig.	Sig.	Sig.
(Dawson *et al.*, 1999)	NDVI	Sig.	Sig.	Sig.
	SAVI	Sig.	Sig.	Sig.
This Study	GP/RT	N/S	Sig.	N/S
	652/605	N/S	N/S	N/S
	GP/990	Sig.	Sig.	Sig.
	990/RT	Sig.	Sig.	Sig.

N/S indicates a statistically non-significant difference between treatments at $p<0.05$.

5.7 Conclusions

The hypotheses investigated in this study were:

Hypothesis 1: *The canopy spectral response of non tolerant grass on clean soil differs from the same grass on contaminated soil.*

Hypothesis 2: *The canopy spectral response of tolerant grass* ***does not*** *alter with levels of contamination that affects the spectral response of non tolerant grass.*

The main reason for measuring chlorophyll content directly was to provide an independent estimate of plant stress. No other techniques exist for independently measuring the stress of grass canopies. According to the chlorophyll concentration results, the addition of metal caused non-tolerant plants to be more stressed than untreated plants only on Sep. 16th. As such it cannot be certain that on other dates there was a stress effect of the metal, it has to be implied that on all other dates the plants were experiencing stress based on the dosage of metal received (section 5.2.3).

Chlorophyll analysis showed little corroboration with the remote sensing results. Where chlorophyll concentrations decreased, there was not necessarily any concomitant significantly different reflectance response. There was no significant change in chlorophyll content over time, while almost every remote sensing technique detected a difference. This could be because the method of chlorophyll analysis itself could be deficient, or factors other than chlorophyll content could have a predominant effect on reflectance. It is not possible to separate these causes with this data, although given the widespread use of the chlorophyll analysis technique used here it is likely that other plant optical features are affecting reflectance (e.g. canopy architecture, intercellular air spaces etc.).

The mixture experiments may have an additional cause for lack of corroboration of chlorophyll concentrations with remote sensing results. The similarity in chlorophyll concentrations between treatments was probably due to the mixture of tolerant and non-tolerant leaves. The remote sensing response to treatment could be due to stressed plants having a greater influence on reflectance, perhaps due to wilting exposing more of the stressed leaves than the still erect tolerant leaves (this was not observed).

The lack of success of indices taken from the literature in detecting the effects of stressors between treatments in the monoculture plots highlights the deficiencies of empirically developed indices, in that they may not be transferable to other situations and/or species. The same is of course true of the indices developed in this study, and until they are tested in other situations they cannot be recommended. The success of the indices in differentiating treatments in the mixture plots, and differentiating results for different dates for the same treatment in monoculture and mixture plots indicates their usefulness in some situations. However, unrealistically extensive research would have to be carried out to identify the responses of ecotypes and species in mixtures and over time.

The red edge position (REP) is perhaps the most commonly tested chlorophyll (and so stress) detection technique for remote sensing. However, for the monoculture data set on 16th September there was a significant change in chlorophyll concentration between the non-tolerant control and high zinc treatment, but no significant change in red edge position (REP) between treatments. For the final monoculture data (15th October) there was no significant change in chlorophyll concentration but there was a significant change in REP between treatments. This indicates that REP is not determined solely by chlorophyll concentration. The inconsistent response of REP to metal input, only responding in the monoculture plots on 15th October, and the mixture plots on 22 October to low zinc and not high, indicates limits in its usefulness in field studies.

In these studies, the tolerance of the plants determined their response to stress, and the stage in the lifecycle that remote sensing measures were taken were crucial in differentiating stressed plants from non-stressed. Results gathered later in the year showed less difference between metal treatments than those from Sep. 16th, despite there being more metal added. Stress effects on plants were apparently not seen directly, instead seasonal changes in plant optical properties affected stressed non-tolerant plants earlier than tolerant or non-stressed non-tolerant plants (Section 2.4.3; Figure 5.74). Thus the stage of lifecycle of the plant is apparently influenced by stress, with stress initiating earlier senescence. If remote sensing is carried out too early in the season, there may be no differences between stressed and non-stressed plants (this may not be the case with very highly stressed plants). If remote sensing is carried out too late in the season all plants may show similar reflectance again as all canopies will be in the final stages of senescence (Fig. 5.74, Schwaller, (1985)). These results thus indicate that timing for remotely sensing contamination expressed through vegetation stress, is critical.

Non-tolerant plants only showed a response to stress which resulted in their earlier senescence, which could only be detected when they were senescing, and non-stressed plants were not. This suggests that to find non-tolerant plants on stressed ground a control area (i.e. known non-stressed area) would first need to be identified. Responses over the target areas to be investigated must then be related to responses over the control area in order to determine if they are senescing early relative to the control. The timing of measurements must also be such that the stressed plants are senescing while the non-stressed plants are either not senescing, or are in the early stages of senescing. This timing may change from year to year, and may also change on different sites with different environmental conditions (Hypotheses 1 accepted with these provisions). Tolerant plants showed no response to stress (Hypotheses 2 accepted).

Ignoring tolerance, remote sensing faces considerable challenges in locating metal contaminated areas. Considering tolerance remote sensing cannot be reliably used to locate contaminated ground, and tolerance has to be considered.

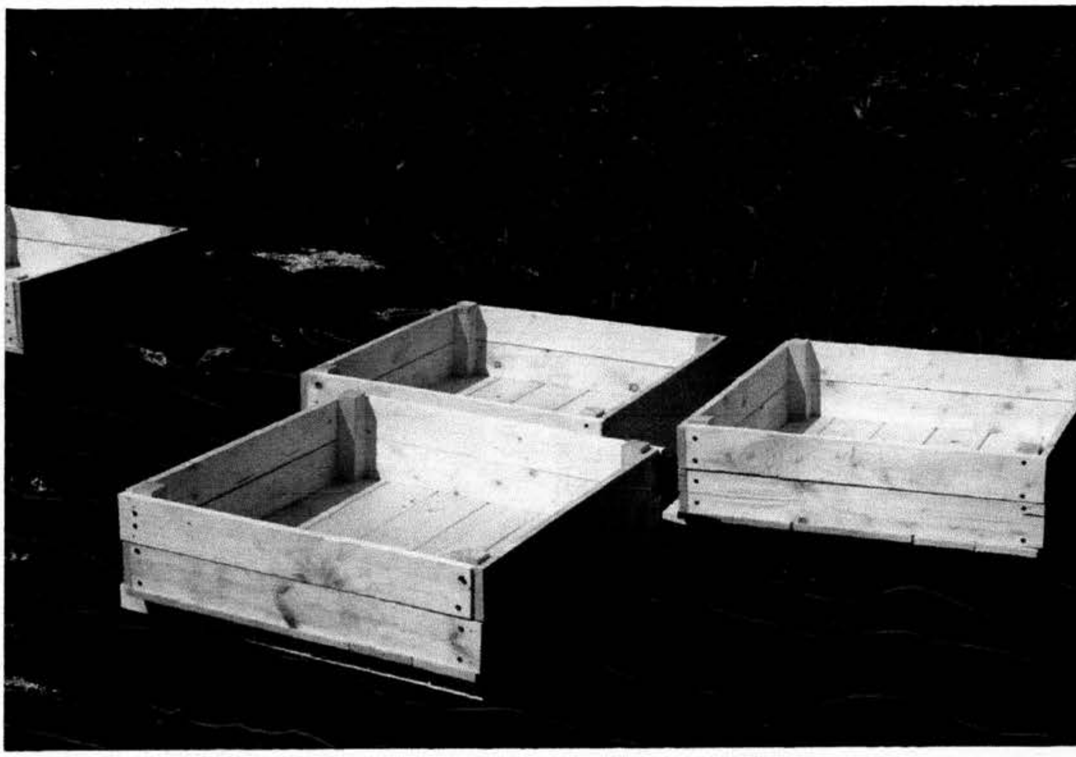

Figure 5.01. Photograph of boxes used to hold soil. These were lined with cloth after this photo was taken.

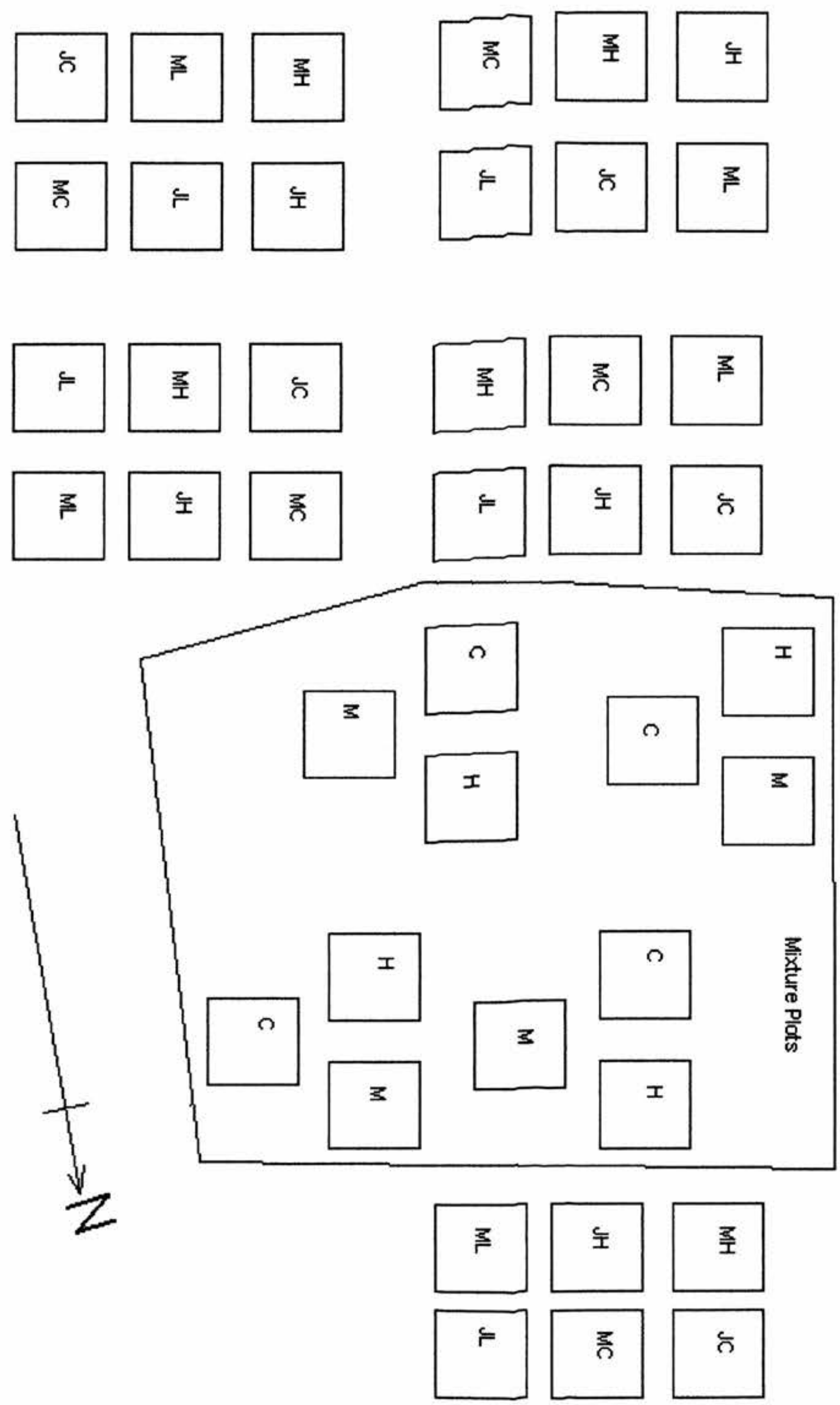

Figure 5.02. Plan of field site showing plot and treatment layout. Monoculture plots were in groups of 6, mixture plots in groups of 3. Monoculture key as in Table 5.02. Mixture codes: C = Control, M = Medium treatment, H = High treatment

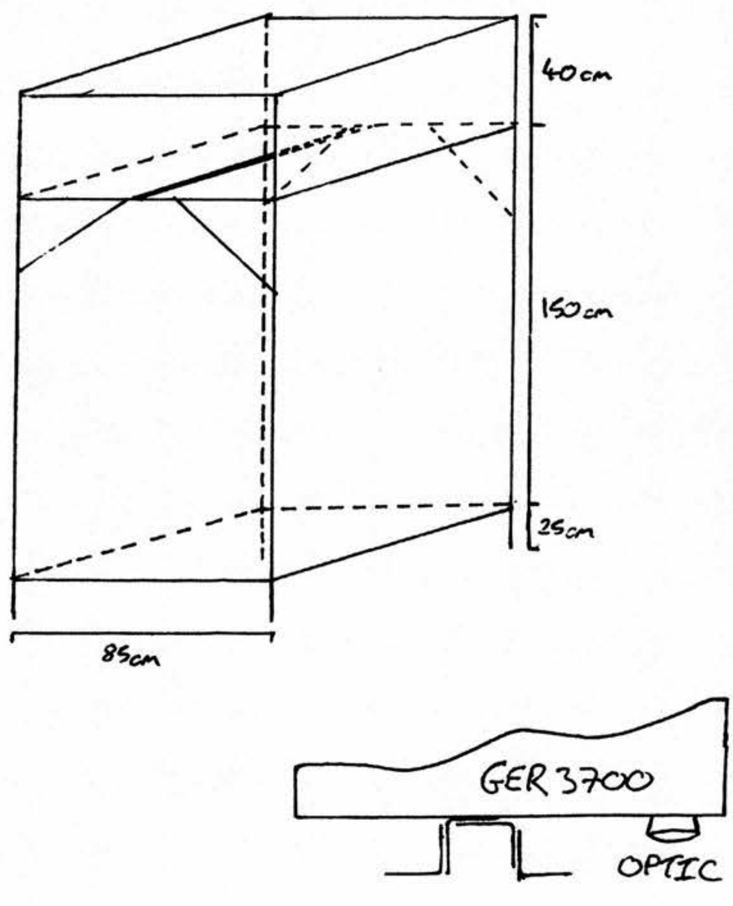

Figure 5.03. Diagram of the frame used to support the spectroradiometer, lights and covers. The spectroradiometer was mounted as shown in the inset onto the central crosspiece 40 cm below the top of the frame.

Figure 5.04. Photograph of the stand with spectroradiometer and lights fitted, but not covered. Some of the experimental plots can be seen in the background.

Figure 5.05. Stand with the spectroradiometer and lights fitted. The matt black cotton inner is being added. In this photo the stand is being used for another study.

Figure 5.06. Tent being used in the field, the spectroradiometer and lights are mounted in the tent, with only the plastic outer covering being seen in this photo. The remote laptop used with the GER3700, and two assistants also in shot.

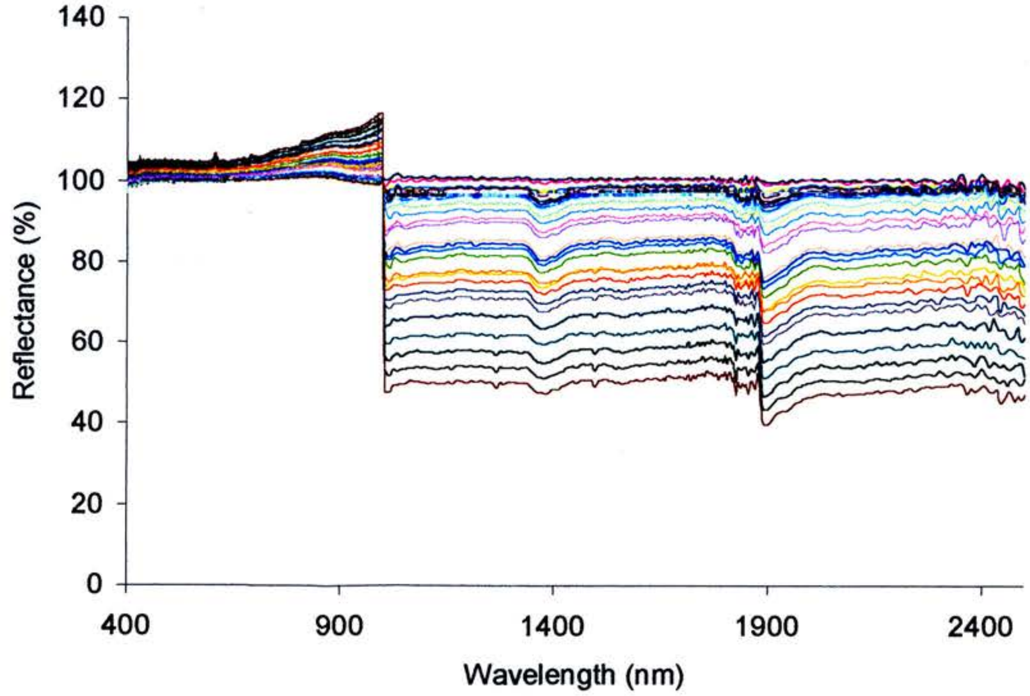

Figure 5.07. Changing reflectance from a constant target over time. Reflectance readings were taken every minute for 30 minutes, and show an increase in reflectance in the visible and a decrease in the infra-red with time.

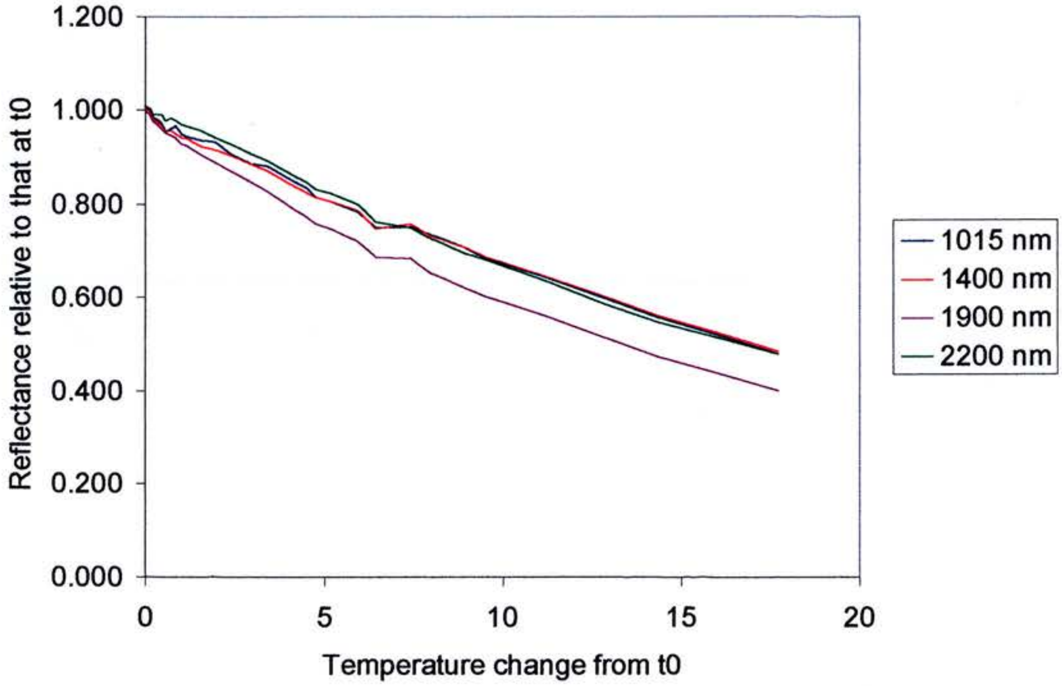

Figure 5.08. The change in reflectance at 4 wavebands across the near infra-red in response to a temperature change in the GER3700.

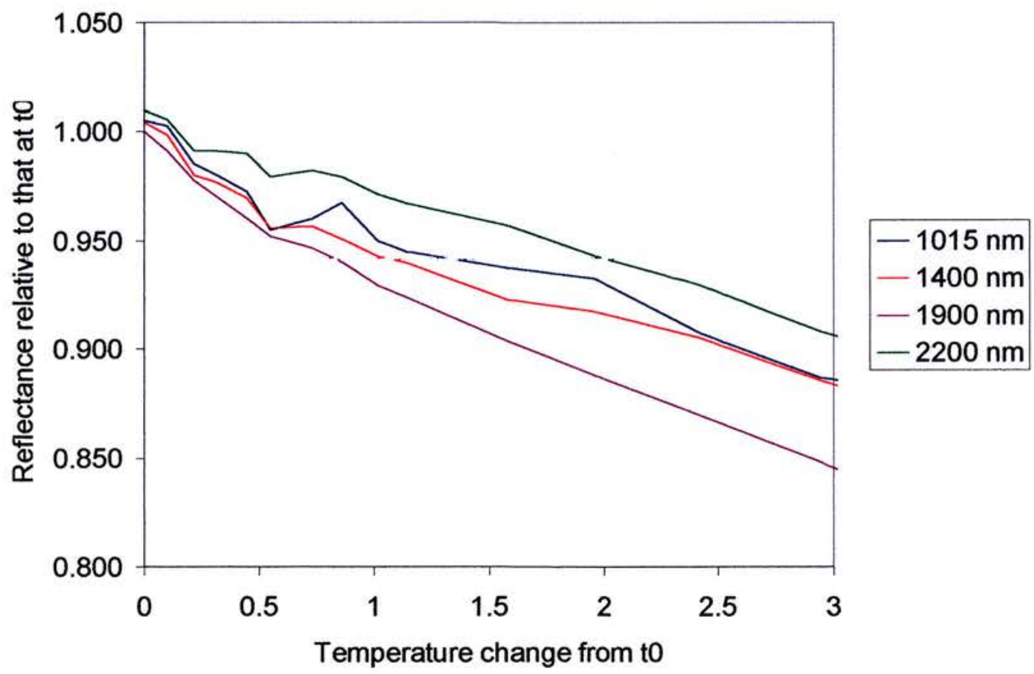

Figure 5.09. How temperature change equivalent to that expected during spectral measurement of each plot affects reflectance at 4 wavebands in the NIR.

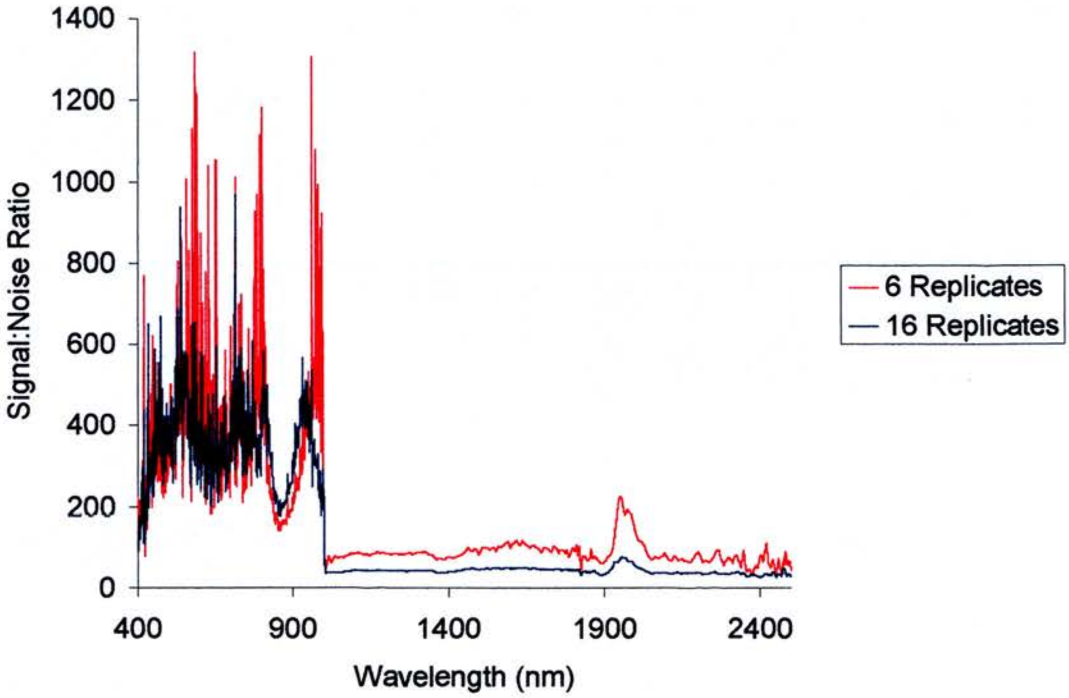

Figure 5.10. Signal to noise ratio using the artificially lit tent with a sequence of 6 and 16 replicate reflectance measurements, using green nylon pads as a target.

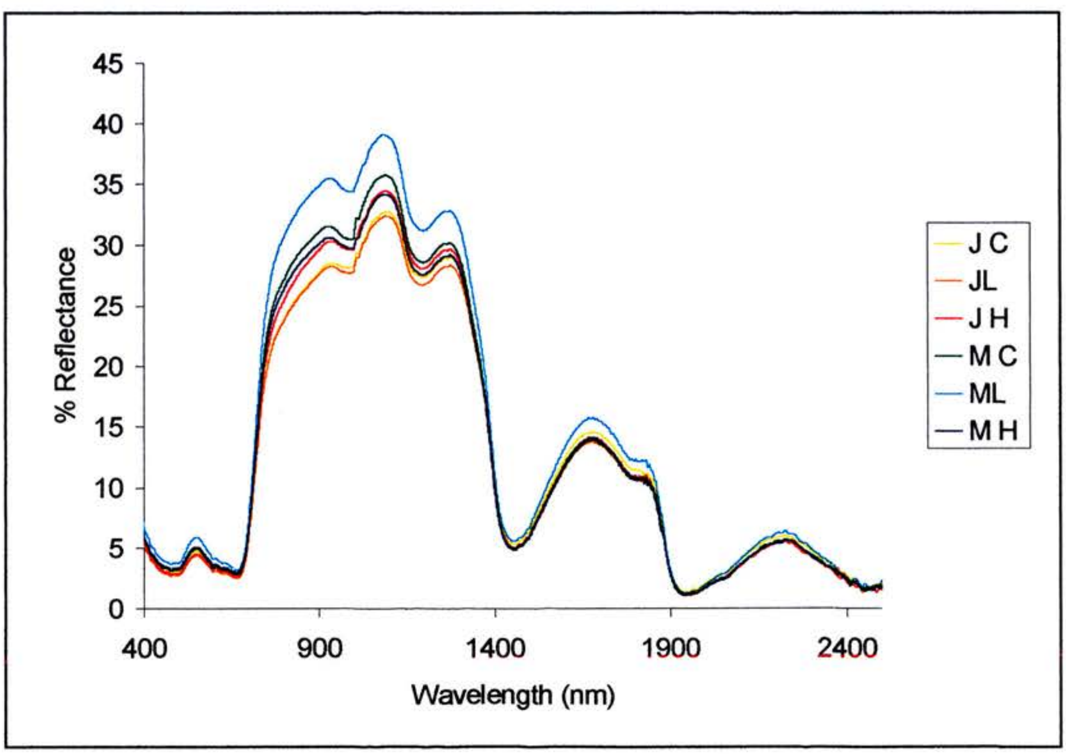

Figure 5.11. Average reflectance response from all treatments Sep 5th.

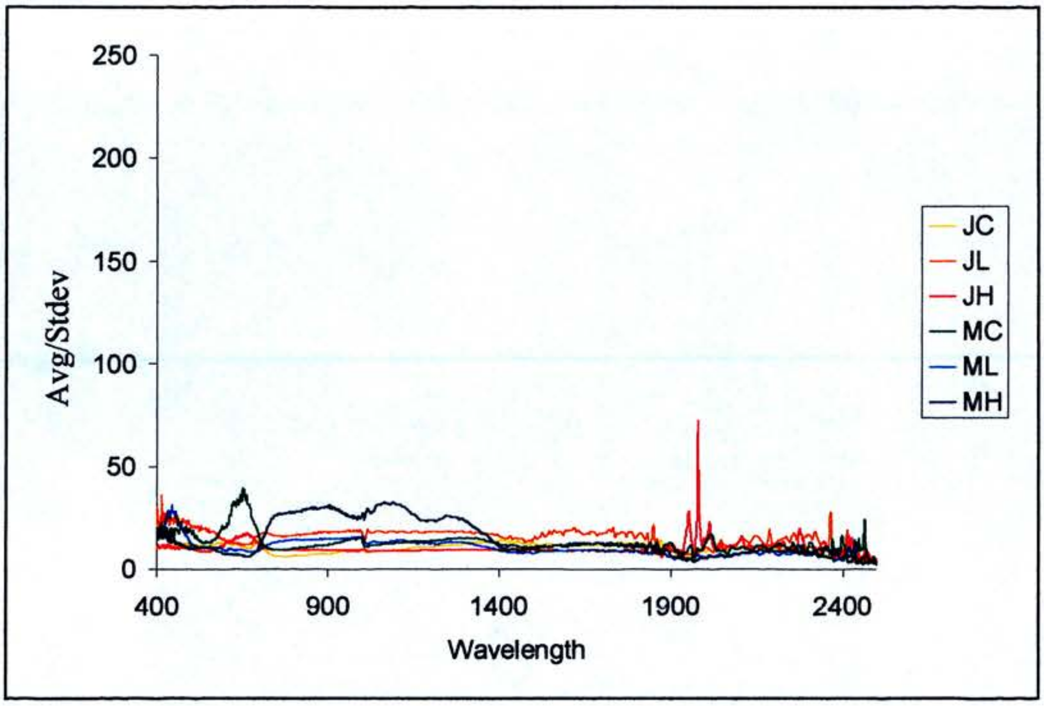

Figure 5.12. Avg/StDev relationship for Sep 5th treatments.

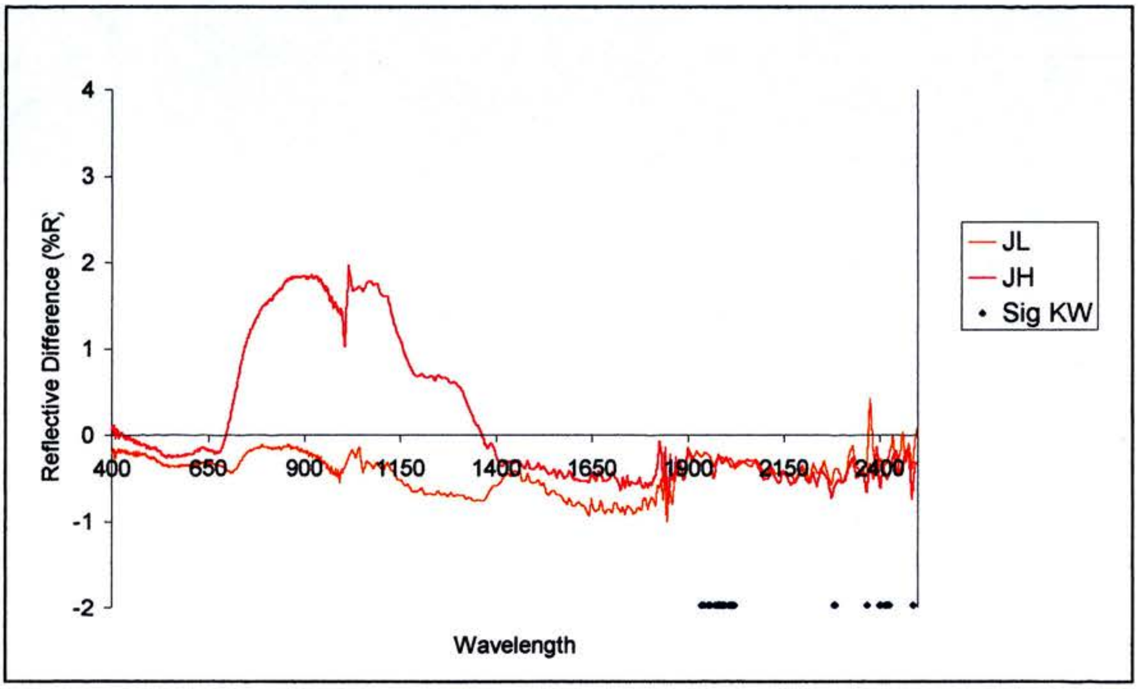

Figure 5.13. Reflectance difference results for *Fr* Jupiter (NT) treatments. "JL" = low zinc, "JH" = high zinc. Statistically different wavelengths also indicated ("Sig KW").

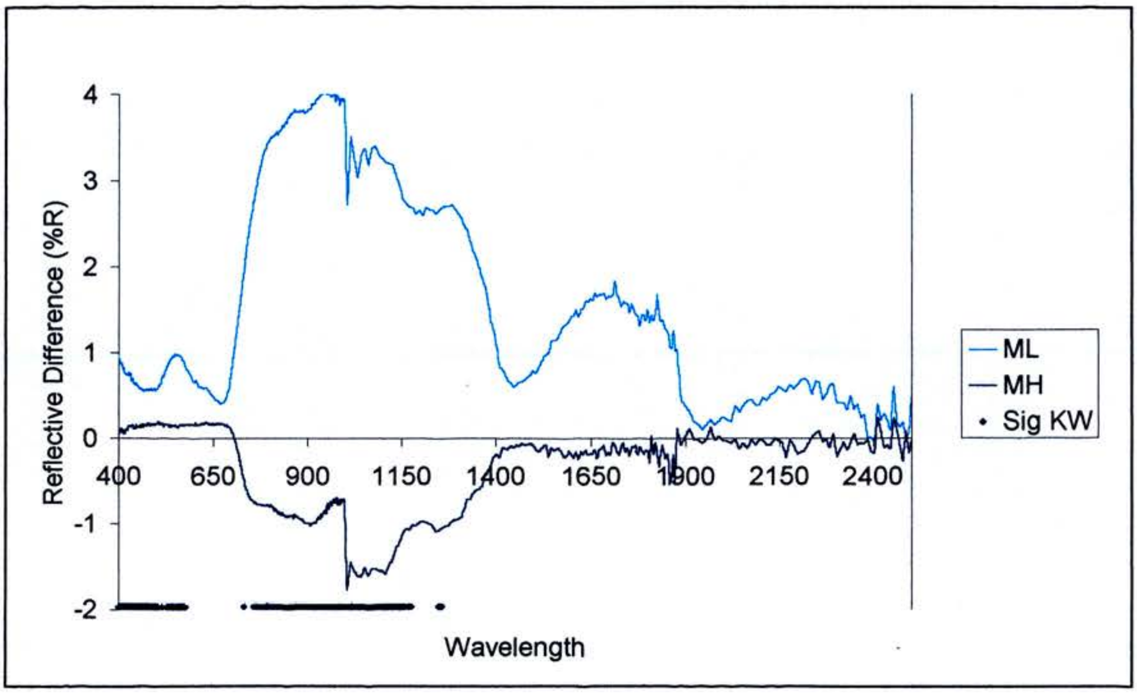

Figure 5.14. Reflective difference results for *Fr* Merlin (T). "ML" = low zinc, "MH" = high zinc. Statistically different wavelengths also indicated ("Sig KW").

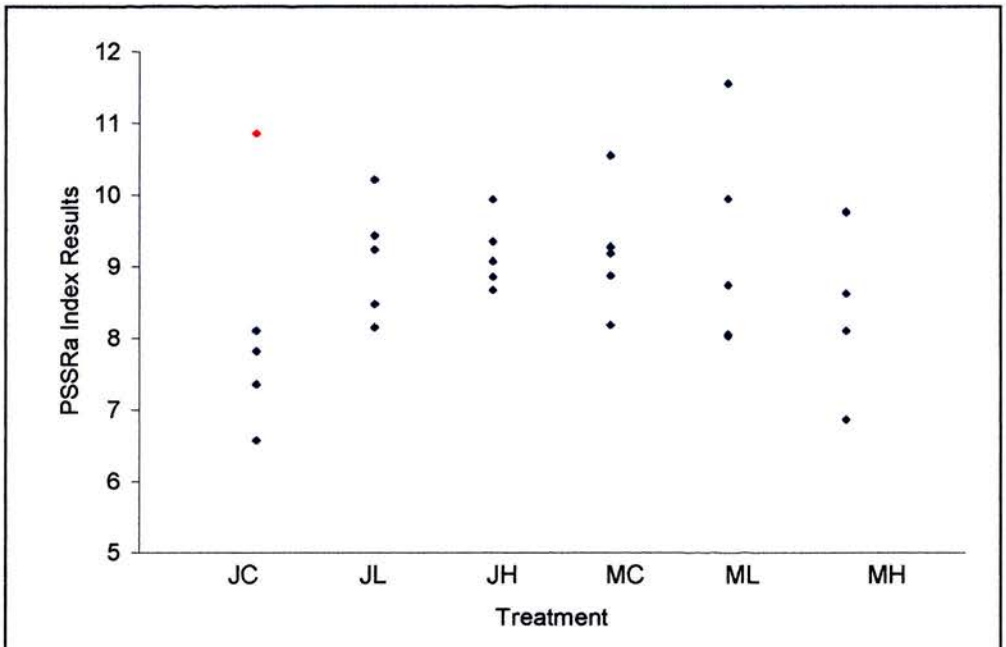

Figure 5.15. Index results for all treatments for Blackburn's PSSRa index. "J" = *Fr* Jupiter (NT) treatments, "M" = *Fr* Merlin (T) treatments, "C" = control, "L" = low zinc, "H" = high zinc. JC would be significantly different from JH if the JC index value labelled in red (from plot 9) were within the range of the other JC values.

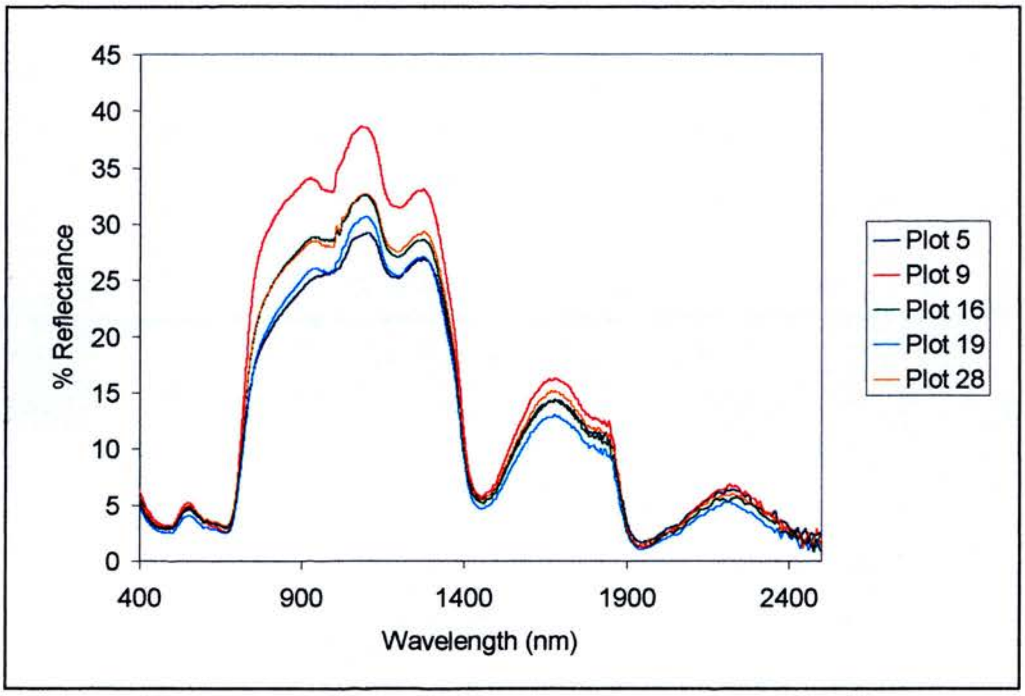

Figure 5.16. Plot reflectance's for the JC treatment showing a different spectral signature in Plot 9 than the other plots.

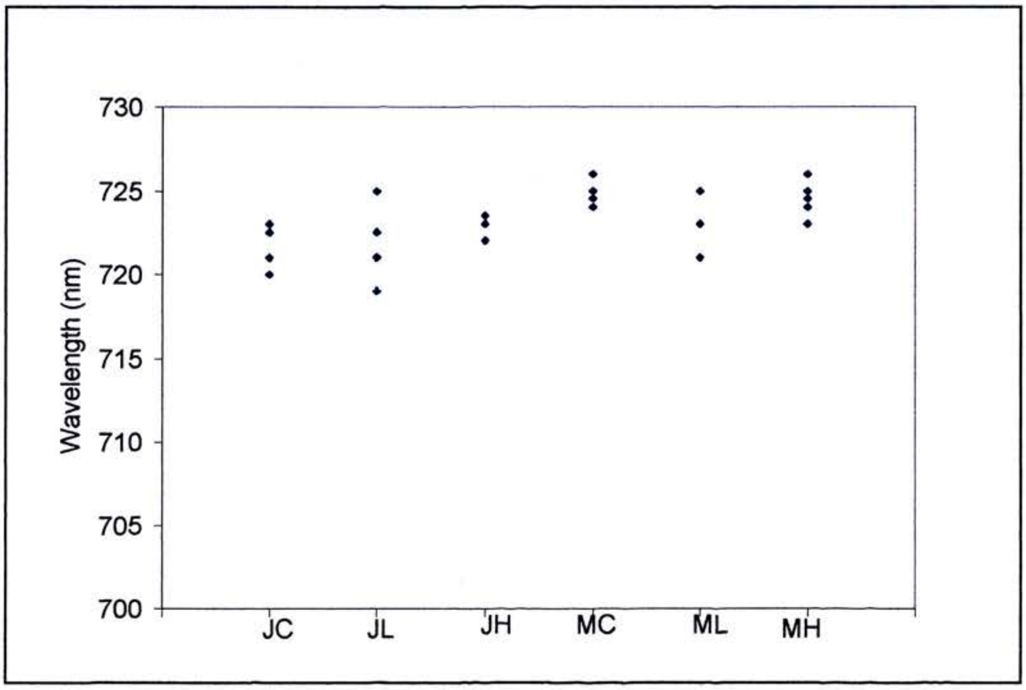

Figure 5.17. Red edge position response to treatment. "J" = *Fr* Jupiter (NT) treatments, "M" = *Fr* Merlin (T) treatments, "C" = control, "L" = low zinc, "H" = high zinc.

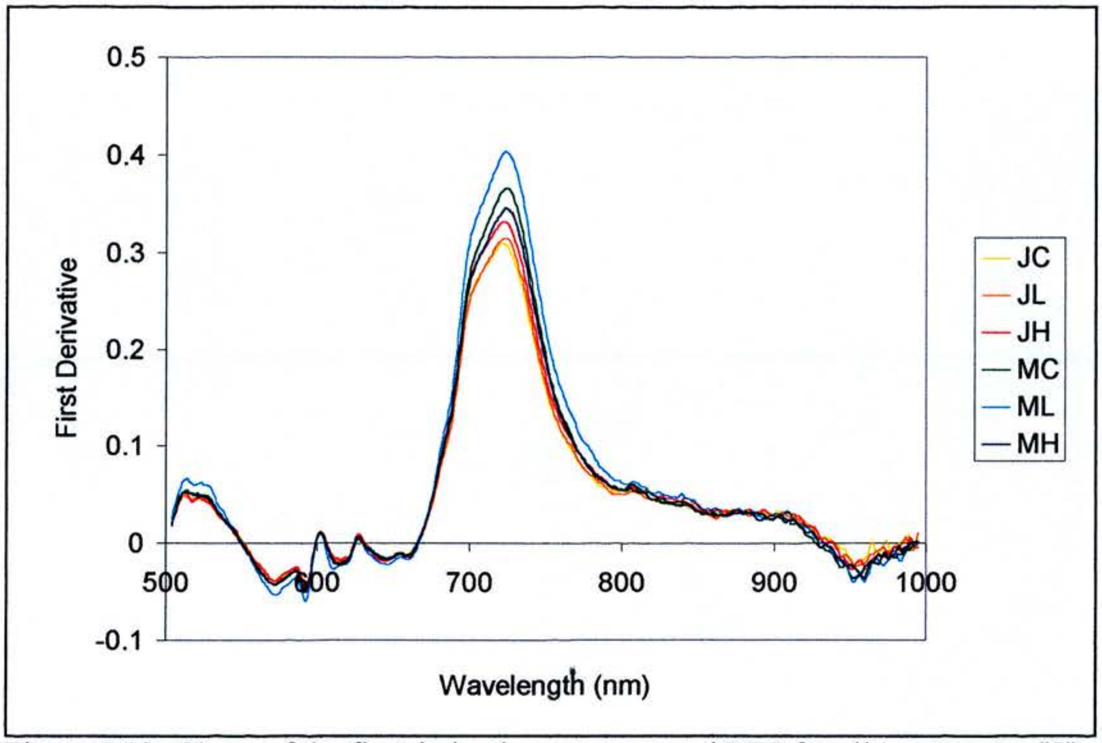

Figure 5.18. Shape of the first derivative curve around REP for all treatments. "J" = *Fr* Jupiter (NT) treatments, "M" = *Fr* Merlin (T) treatments, "C" = control, "L" = low zinc, "H" = high zinc.

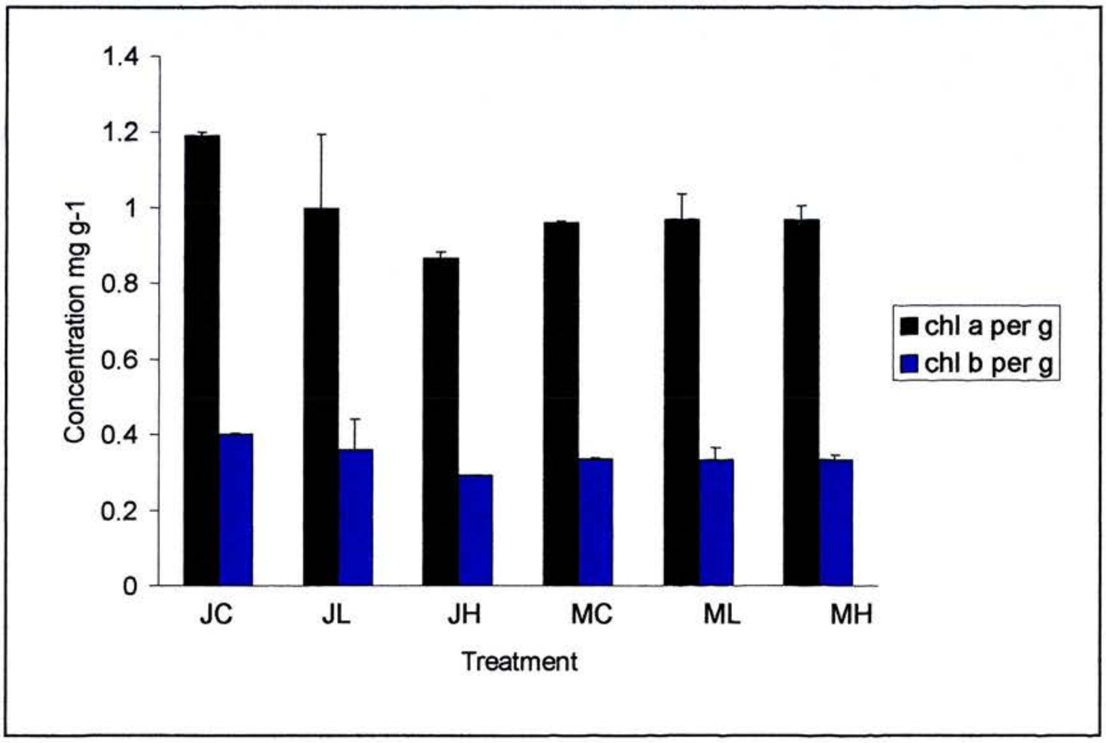

Figure 5.19. Chlorophyll concentrations mg g^{-1} wet leaf weight. "J" = *Fr* Jupiter (NT) treatments, "M" = *Fr* Merlin (T) treatments, "C" = control, "L" = low zinc, "H" = high zinc. Error bars show +1 St.Dev.

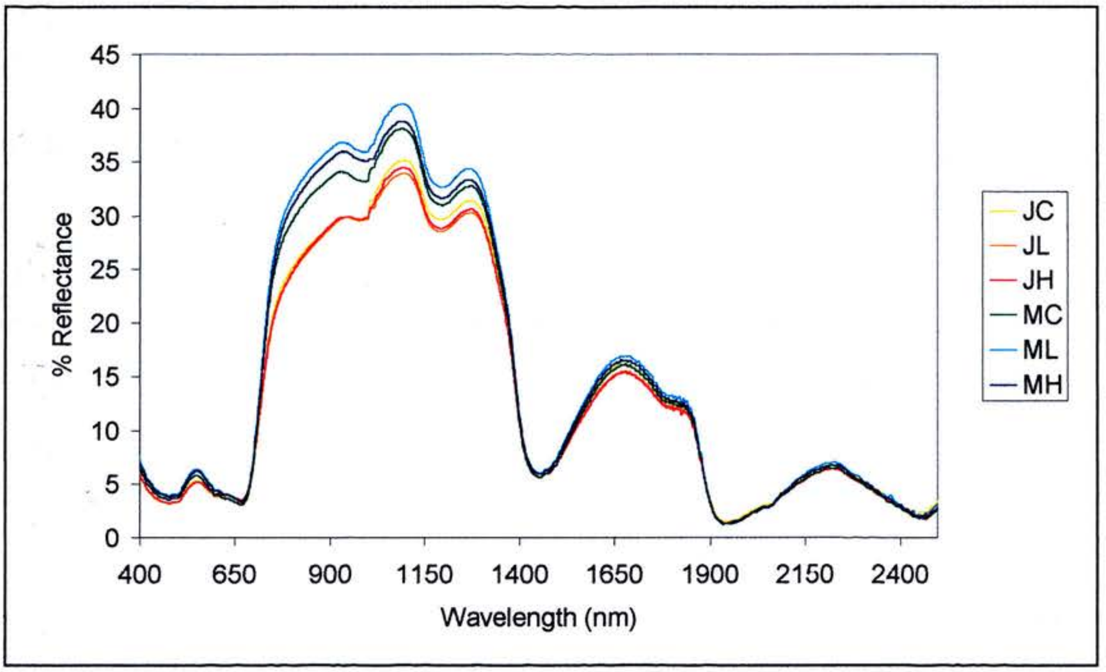

Figure 5.20. Average reflectance for each treatment Sep 16th. "J" = *Fr* Jupiter (NT) treatments, "M" = *Fr* Merlin (T) treatments, "C" = control, "L" = low zinc, "H" = high zinc.

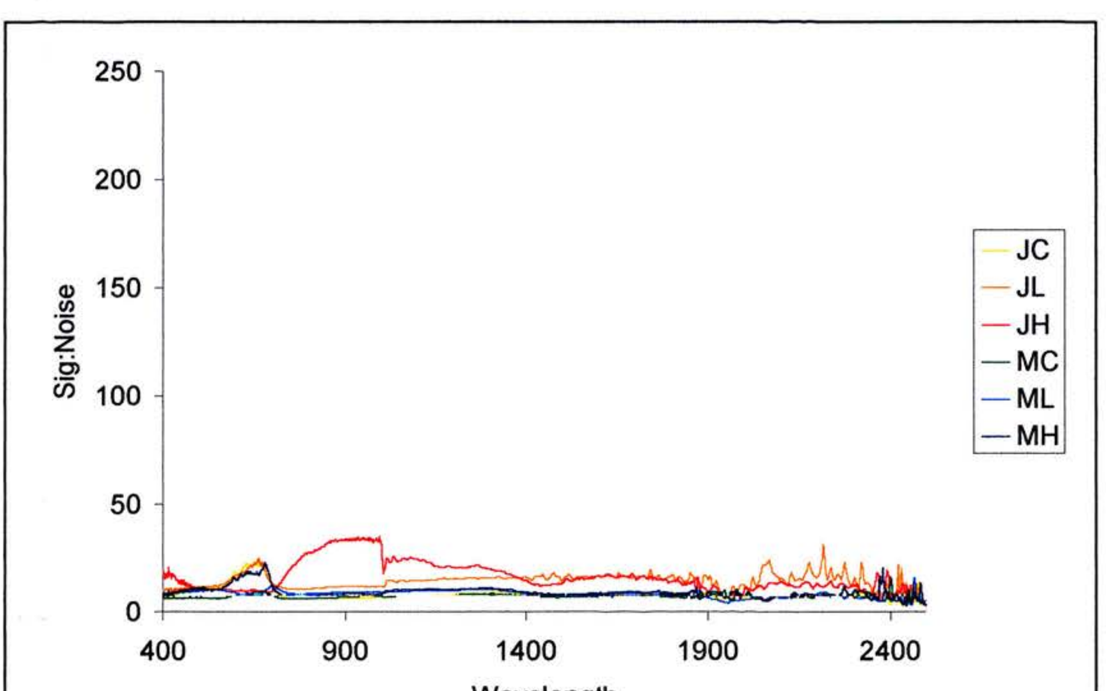

Figure 5.21 Signal : Noise relationships for 16-9 treatments. "J" = *Fr* Jupiter (NT) treatments, "M" = *Fr* Merlin (T) treatments, "C" = control, "L" = low zinc, "H" = high zinc.

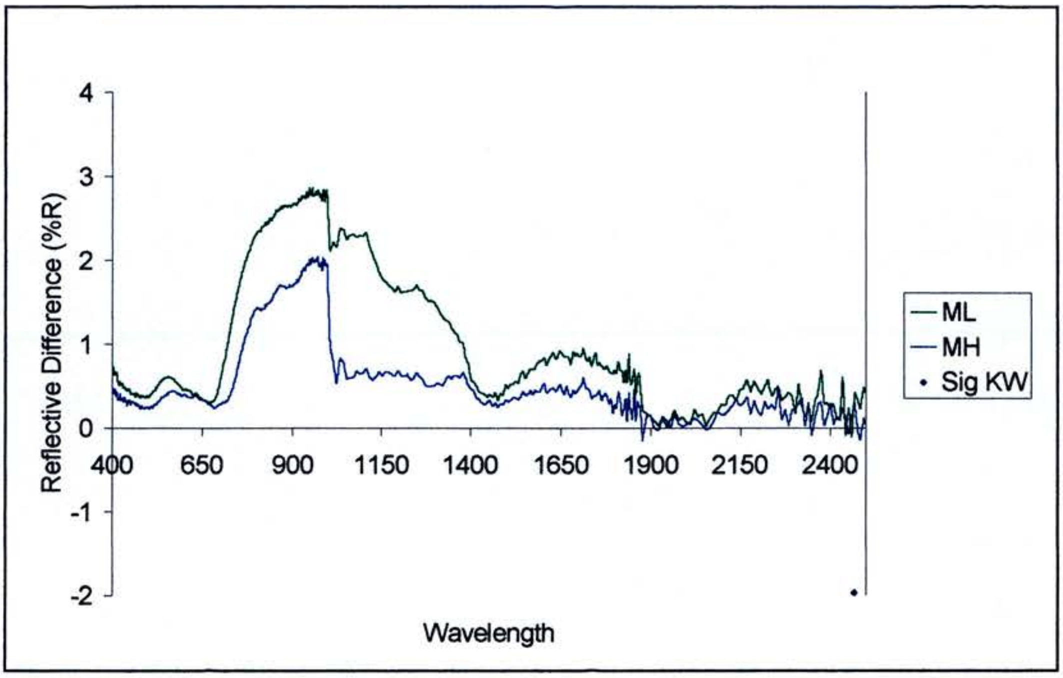

Figure 5.24. Reflectance difference results with associated statistical results for *Fr* Merlin (T) treatments. "ML" = low zinc, "MH" = high zinc. Statistically significant differences are also indicates, "Sig KW".

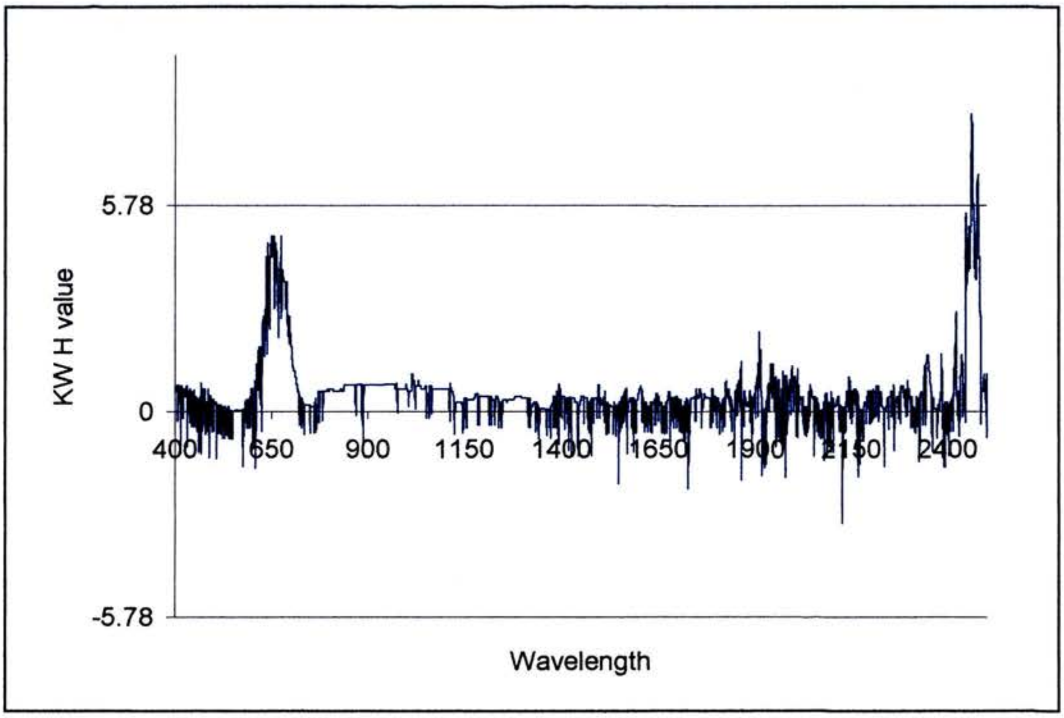

Figure 5.25. Kruskal - Wallis H (KW-H) values for all Jupiter replicates. A KW-H value >5.78 or <-5.78 indicates a significant difference between treatments

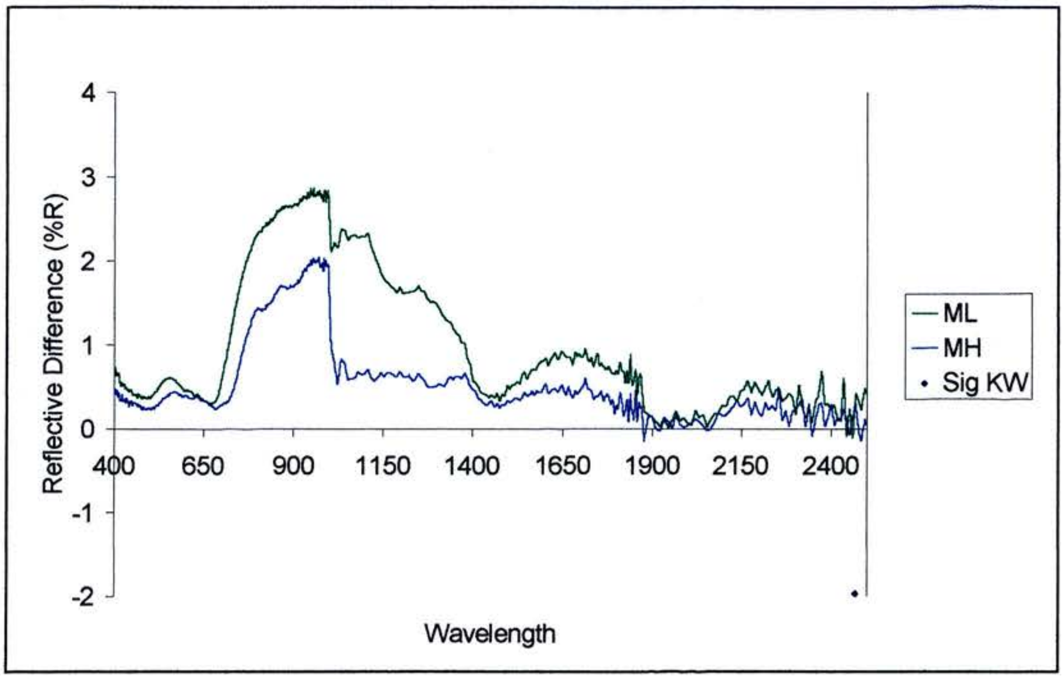

Figure 5.24. Reflectance difference results with associated statistical results for *Fr* Merlin (T) treatments. "ML" = low zinc, "MH" = high zinc. Statistically significant differences are also indicates, "Sig KW".

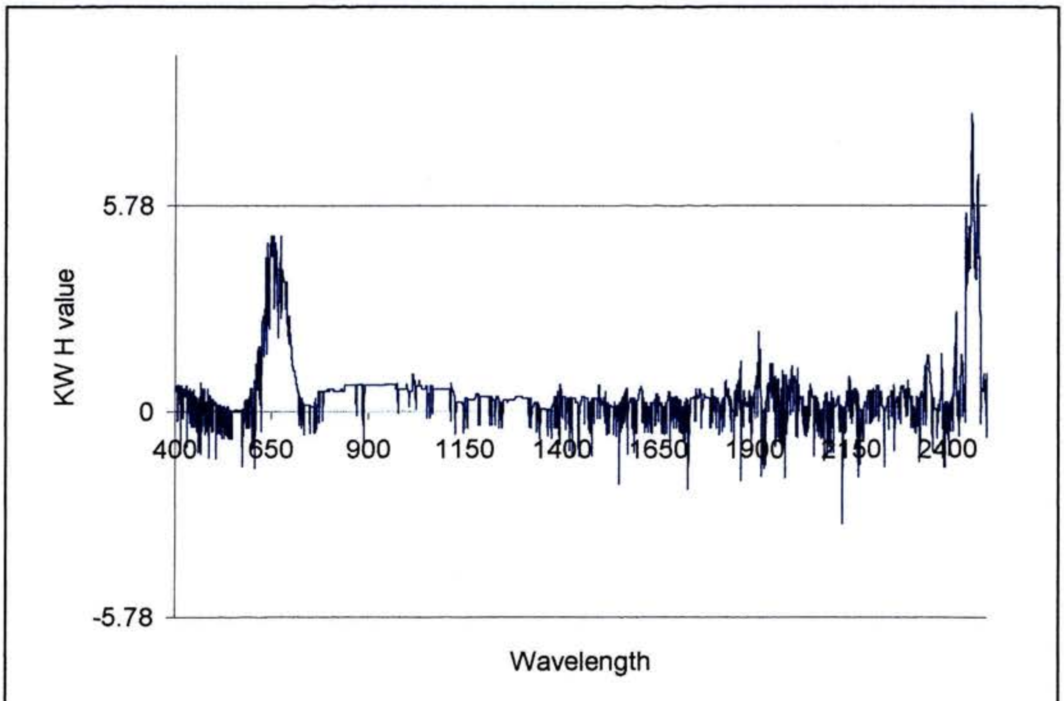

Figure 5.25. Kruskal - Wallis H (KW-H) values for all Jupiter replicates. A KW-H value >5.78 or <-5.78 indicates a significant difference between treatments

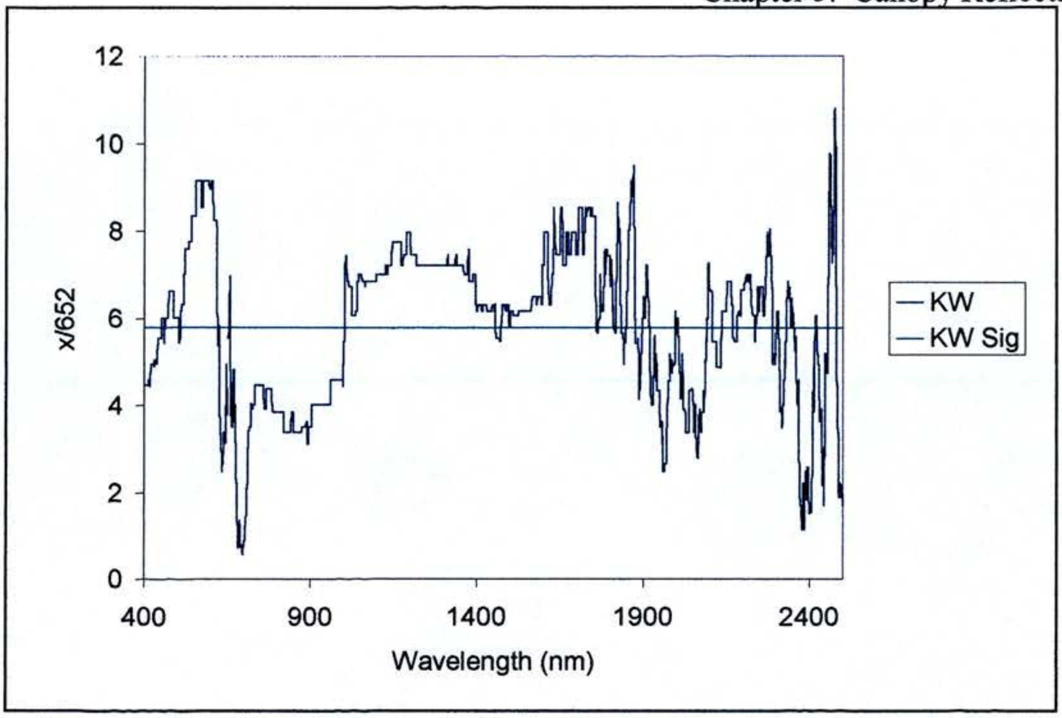

Figure 5.26. Kruskal Wallis H values for indices based on x/652 for *Fr* Jupiter (NT) treatments, where x is the waveband at which the significance of the result is recorded. 600/652 and some NIR/652 give significant results.

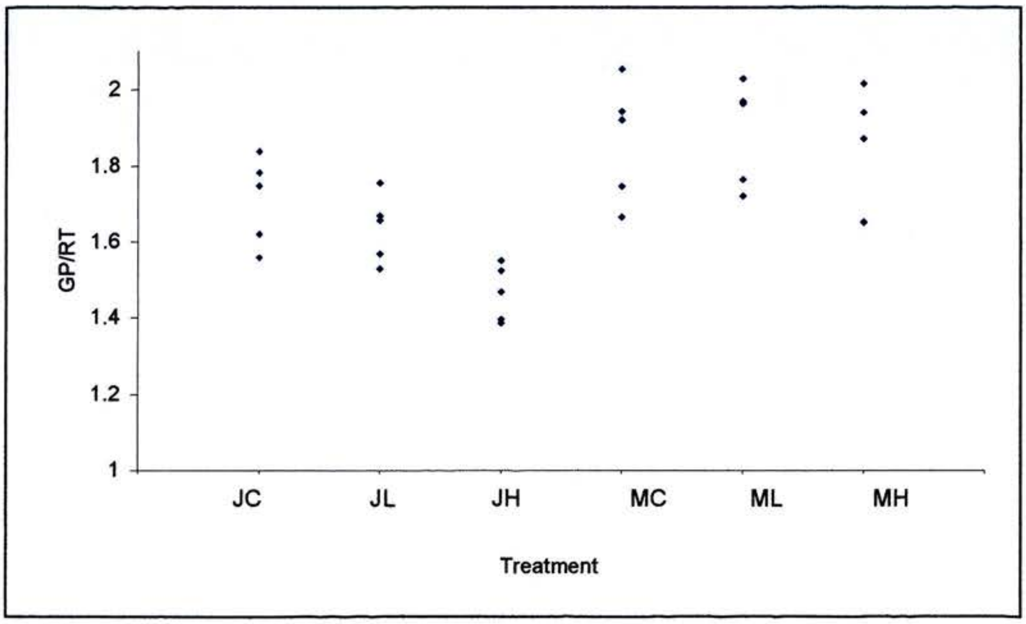

Figure 5.27. Results for the GP/RT (Green Peak/Red Trough) index. "J" = *Fr* Jupiter (NT) treatments, "M" = *Fr* Merlin (T) treatments, "C" = control, "L" = low zinc, "H" = high zinc.

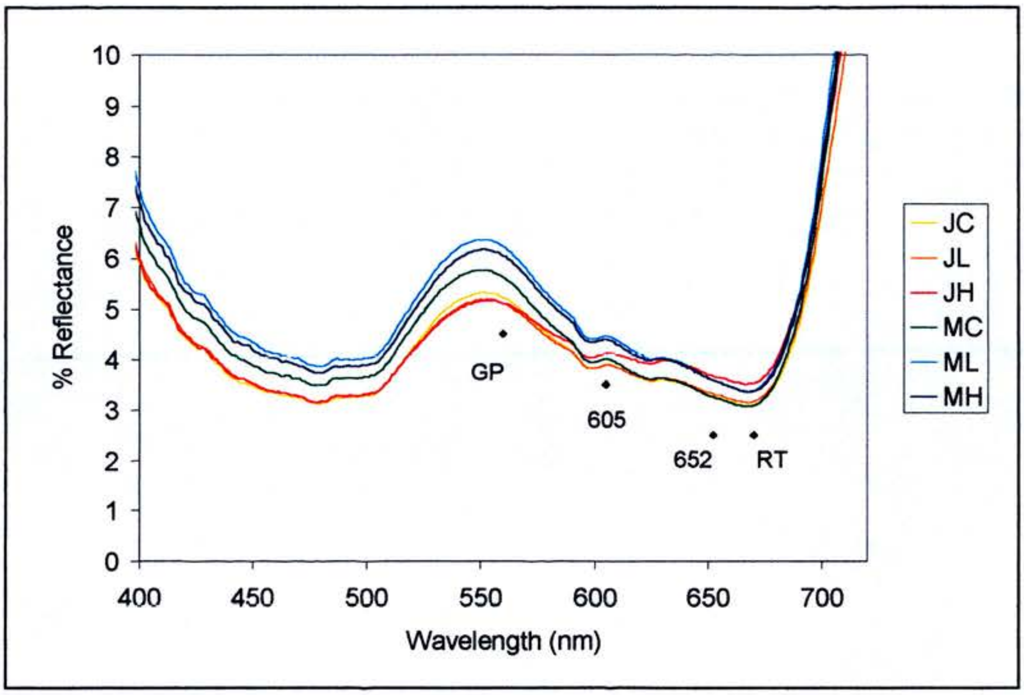

Figure 5.28. Reflectance data with wavebands used in indices highlighted.

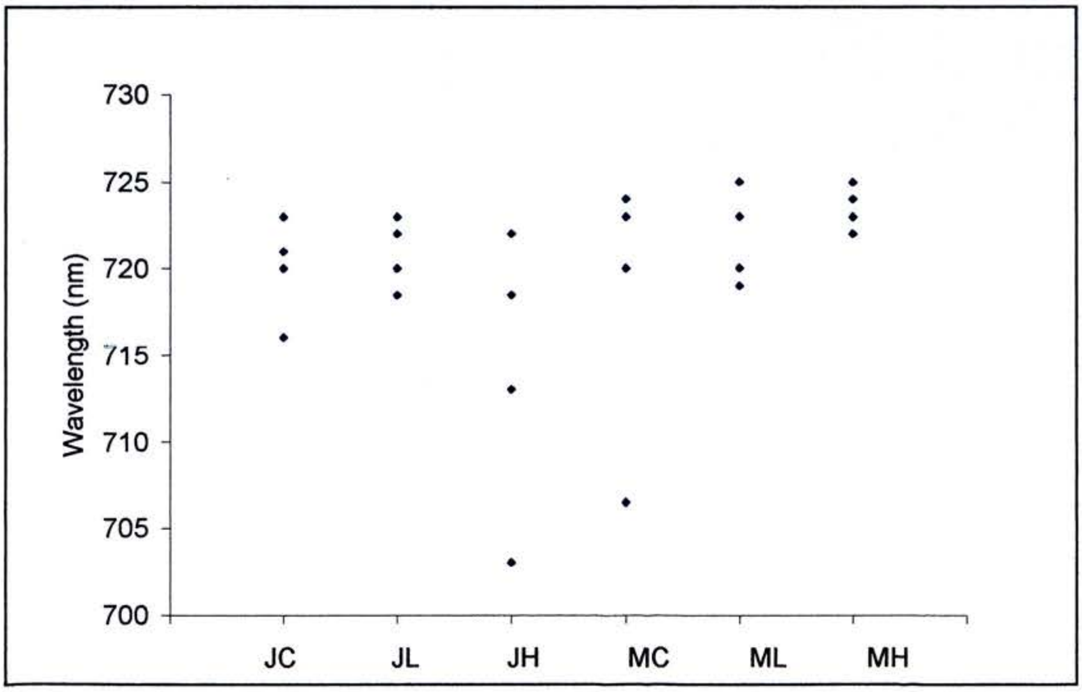

Figure 5.29. Red Edge Position for each replicate of all treatments Sep 16th. "J" = *Fr* Jupiter (NT) treatments, "M" = *Fr* Merlin (T) treatments, "C" = control, "L" = low zinc, "H" = high zinc.

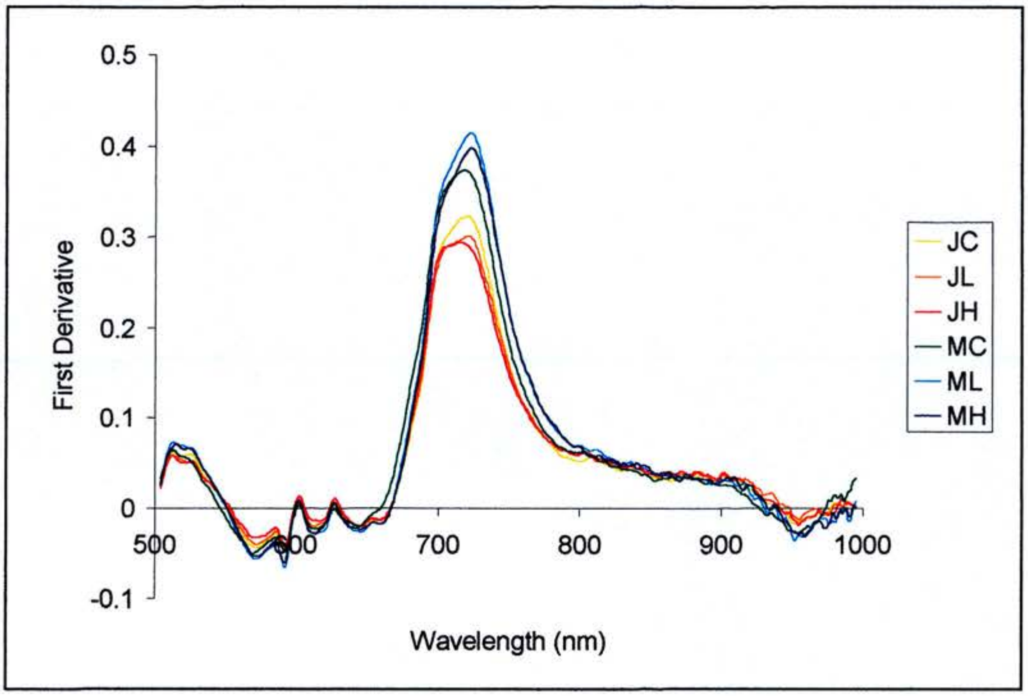

Figure 5.30. First derivative of reflectance around the red edge Sep 16th. "J" = *Fr* Jupiter (NT) treatments, "M" = *Fr* Merlin (T) treatments, "C" = control, "L" = low zinc, "H" = high zinc.

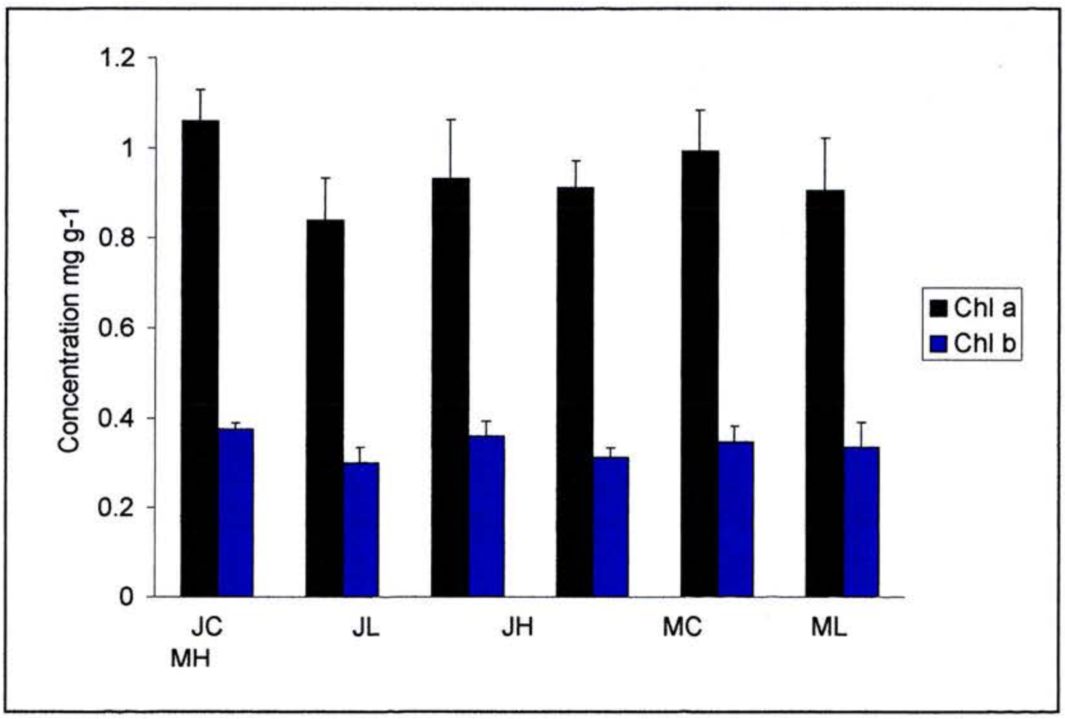

Figure 5.31 Chlorophyll concentrations Oct. 15th. "J" = *Fr* Jupiter (NT) treatments, "M" = *Fr* Merlin (T) treatments, "C" = control, "L" = low zinc, "H" = high zinc. Error bars show +1 St.Dev.

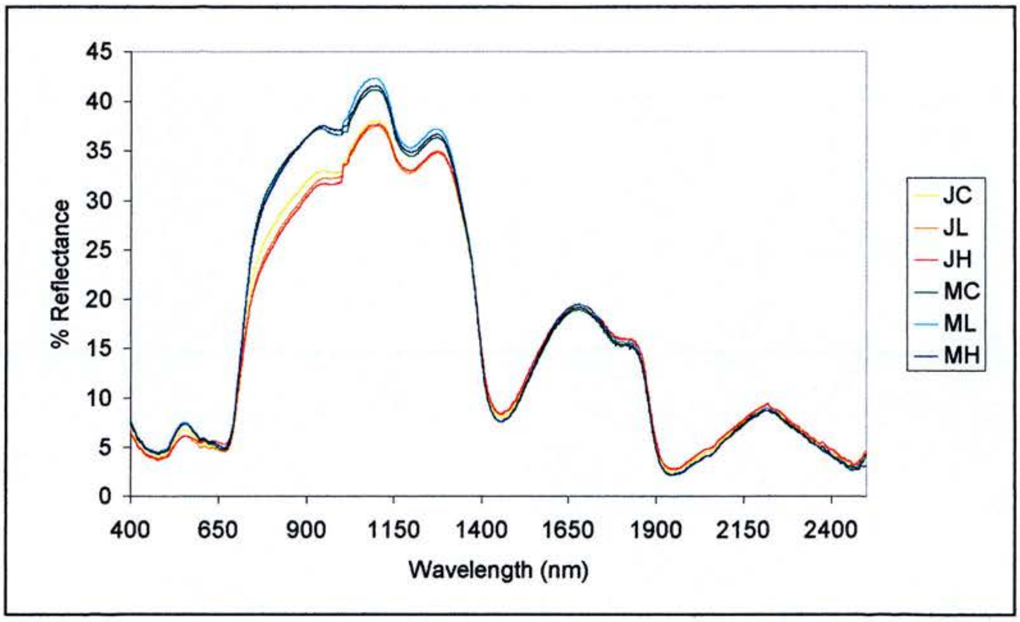

Figure 5.32. Average reflectance response of all treatments Oct 15th. "J" = *Fr* Jupiter (NT) treatments, "M" = *Fr* Merlin (T) treatments, "C" = control, "L" = low zinc, "H" = high zinc.

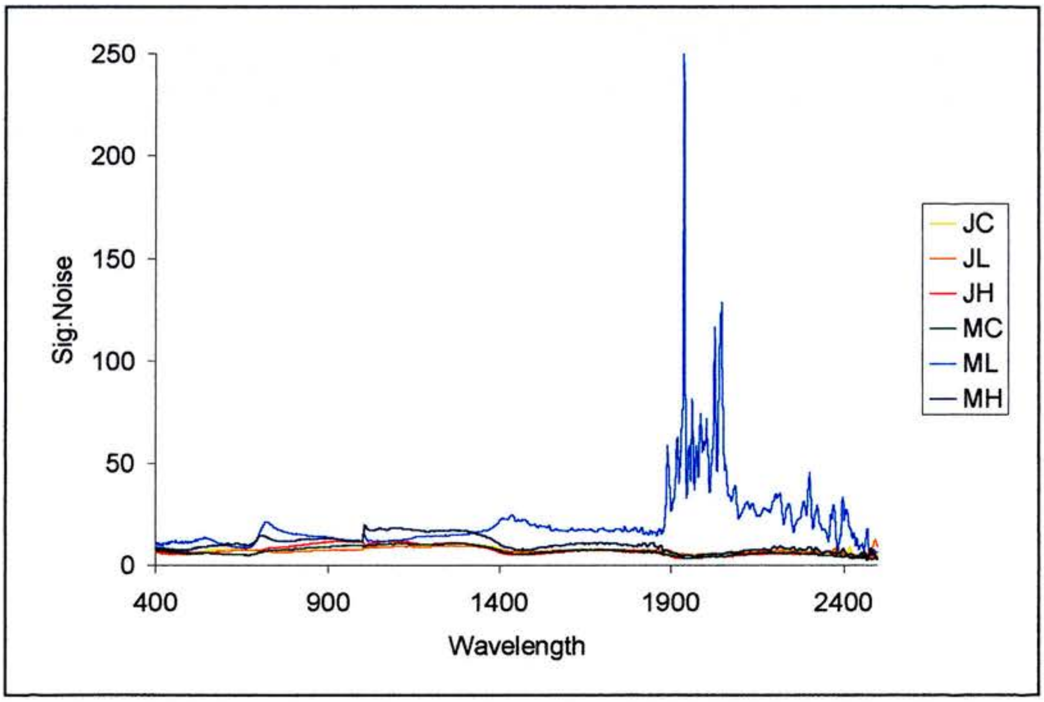

Figure 5.33 Signal : Noise relationship for all treatments, Oct. 15th. "J" = *Fr* Jupiter (NT) treatments, "M" = *Fr* Merlin (T) treatments, "C" = control, "L" = low zinc, "H" = high zinc.

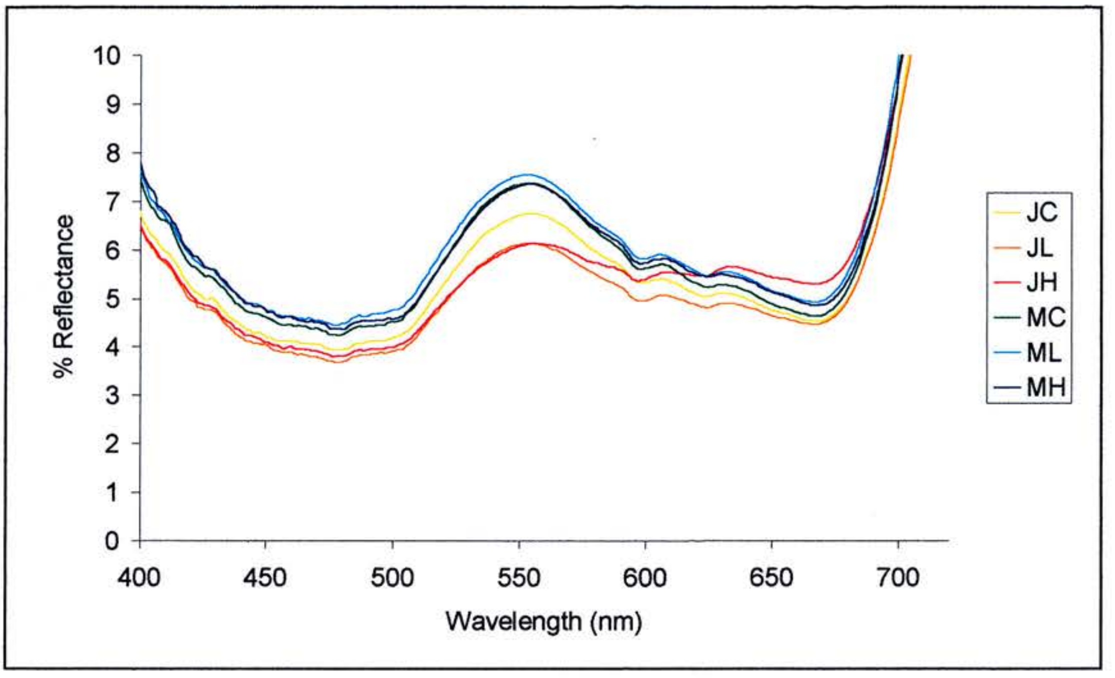

Figure 5.34. Average reflectance results for all treatments in the visible region of the spectrum, Oct. 15th. "J" = *Fr* Jupiter (NT) treatments, "M" = *Fr* Merlin (T) treatments, "C" = control, "L" = low zinc, "H" = high zinc.

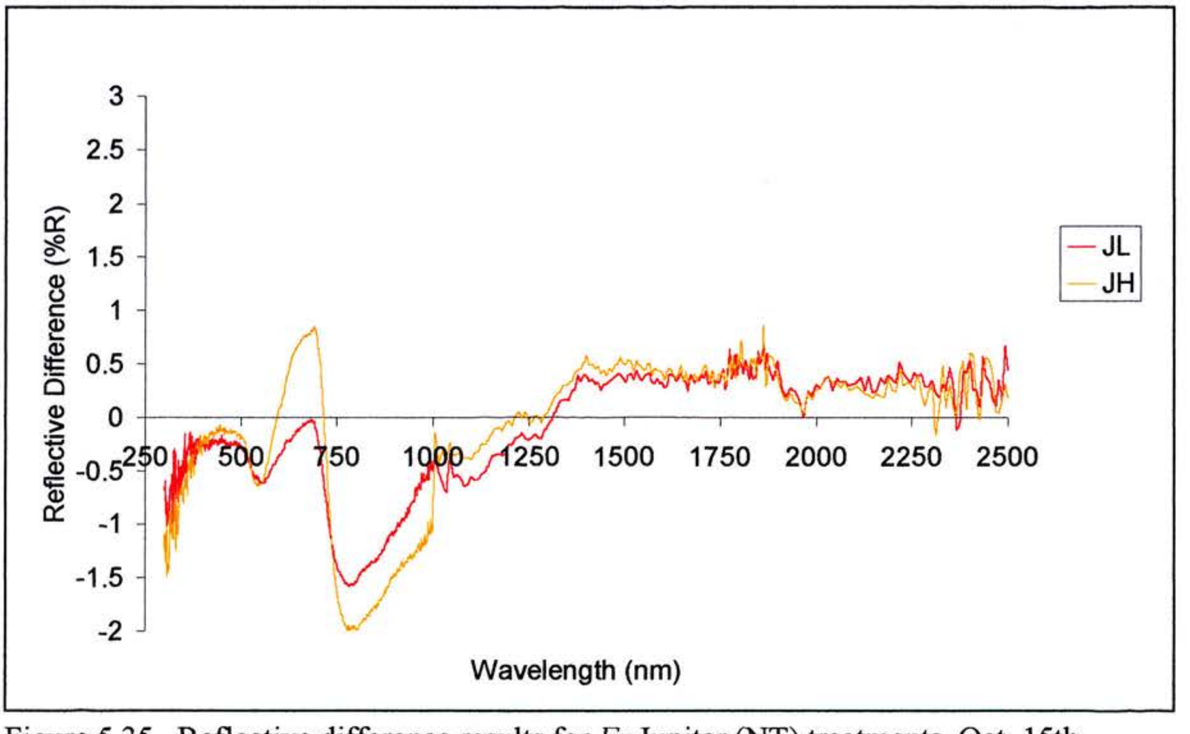

Figure 5.35. Reflective difference results for *Fr* Jupiter (NT) treatments, Oct. 15th. "JL" = low zinc, "JH" = high zinc.

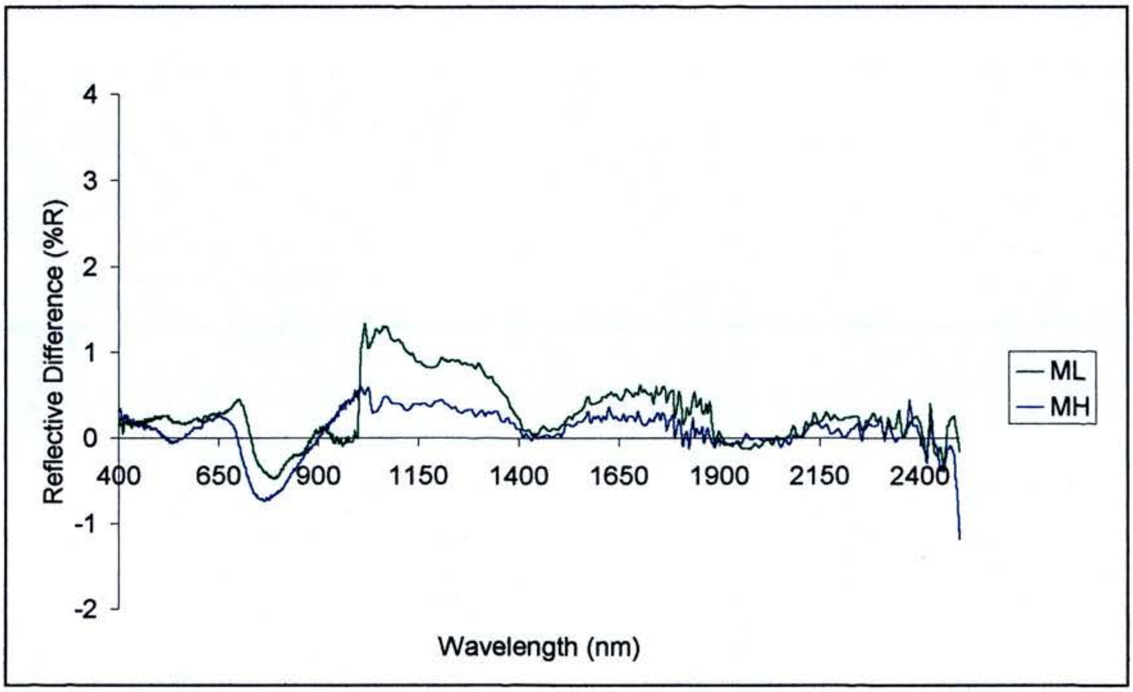

Figure 5.36. Reflective difference results for *Fr* Merlin (T) treatments, Oct. 15th. "ML" = low zinc, "MH" = high zinc.

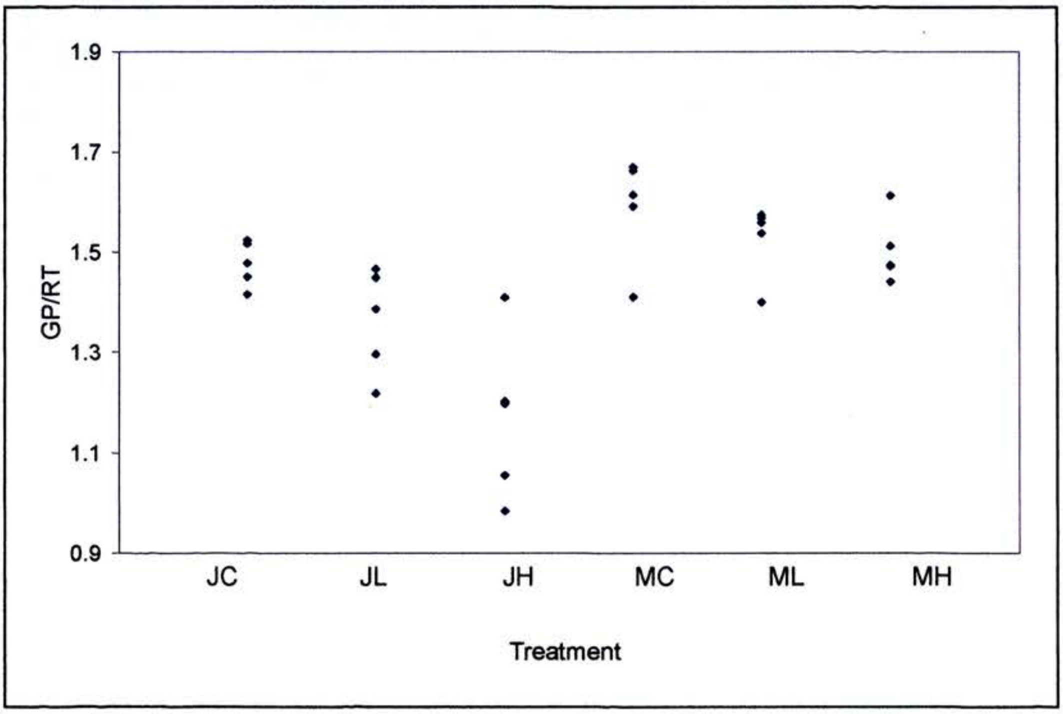

Figure 5.37. Results for Green Peak/ Red Trough (GP/RT) index. "J" = *Fr* Jupiter (NT) treatments, "M" = *Fr* Merlin (T) treatments, "C" = control, "L" = low zinc, "H" = high zinc.

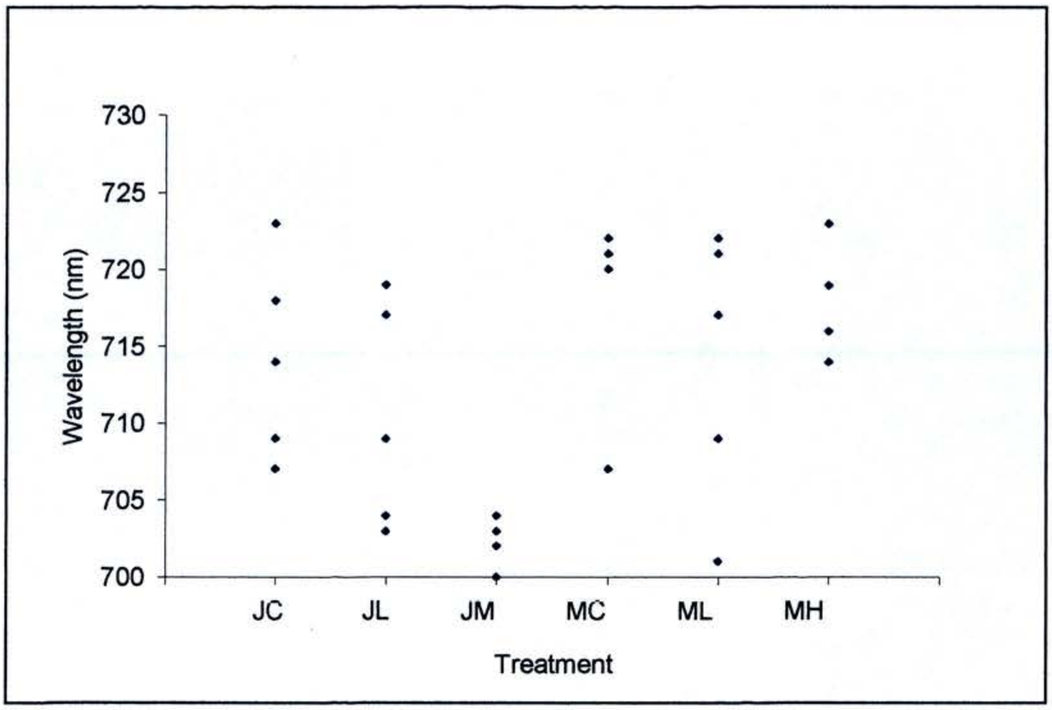

Figure 5.38. Red edge position in response to treatment Oct. 15th. "J" = *Fr* Jupiter (NT) treatments, "M" = *Fr* Merlin (T) treatments, "C" = control, "L" = low zinc, "H" = high zinc.

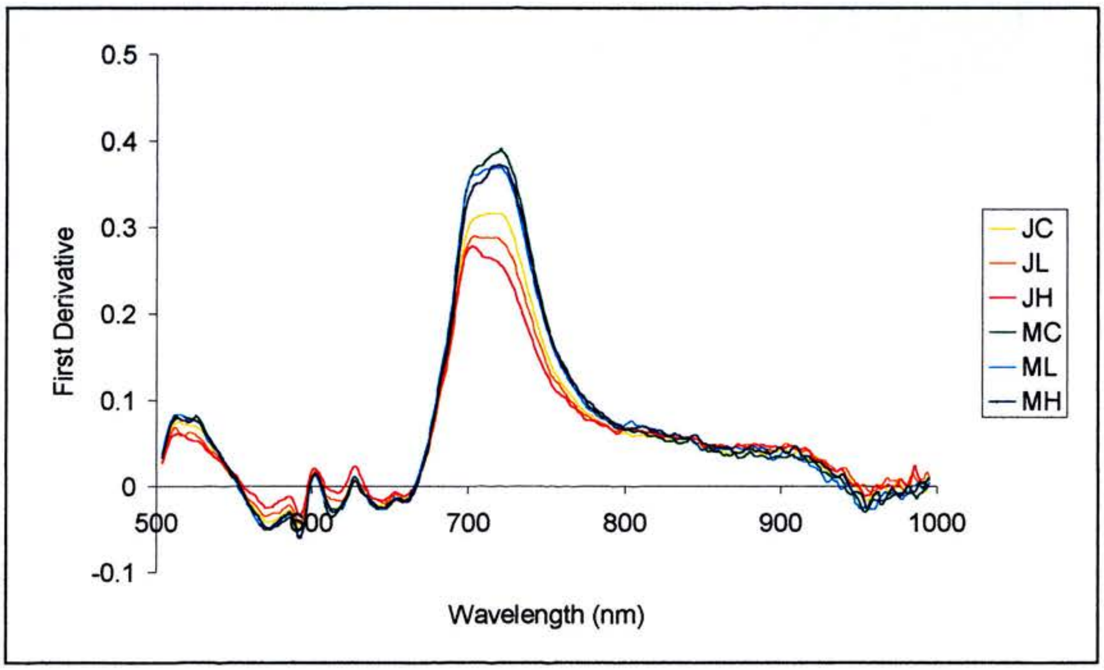

Figure 5.39. Shape of the first derivative of reflectance around the REP for all treatments. "J" = *Fr* Jupiter (NT) treatments, "M" = *Fr* Merlin (T) treatments, "C" = control, "L" = low zinc, "H" = high zinc.

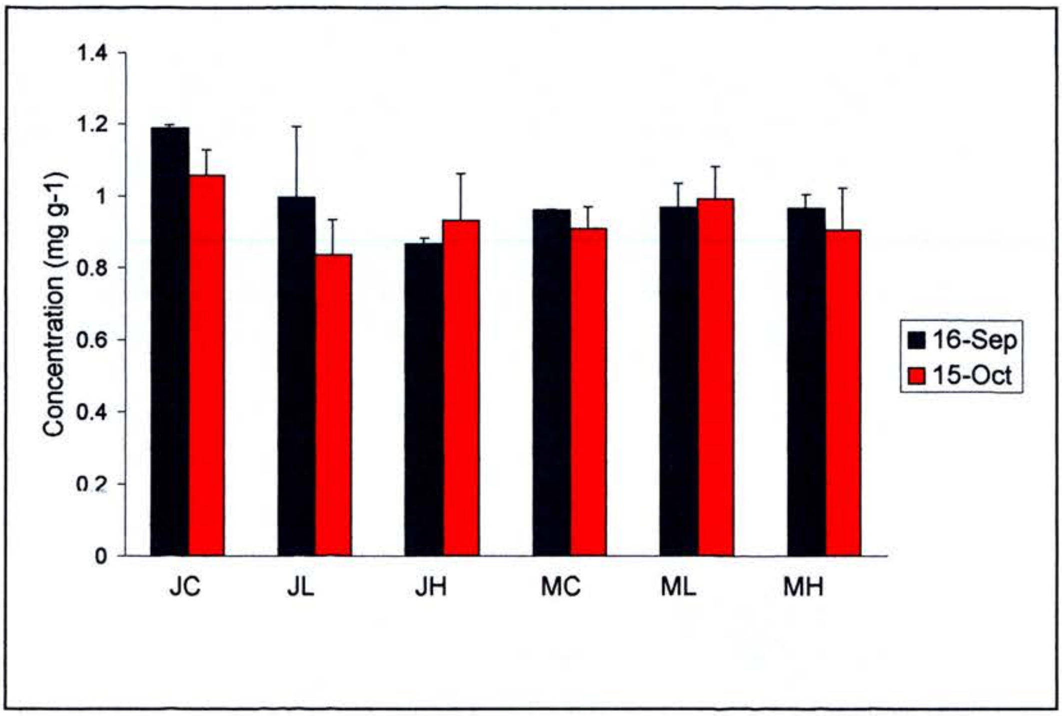

Figure 5.40 Change in chlorophyll concentration over time for all treatments. "J" = *Fr* Jupiter (NT) treatments, "M" = *Fr* Merlin (T) treatments, "C" = control, "L" = low zinc, "H" = high zinc. Error bars show +1 St.Dev.

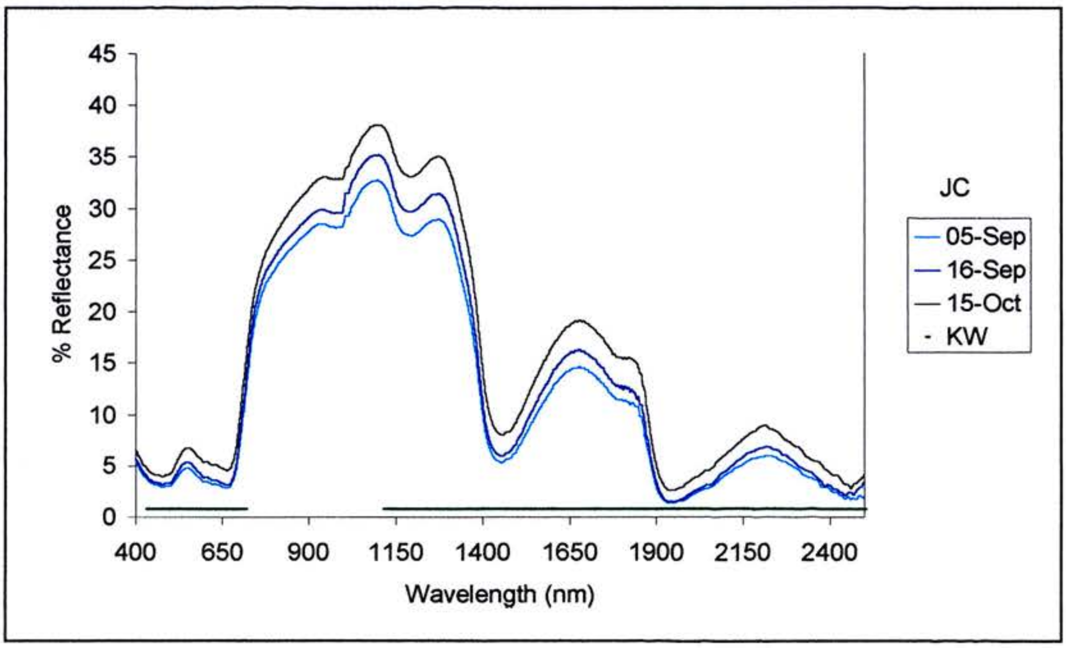

Figure 5.41 . Changing average *Fr* Jupiter (NT) control treatment reflectance with time. Significant differences between dates reflectance is shown ("KW").

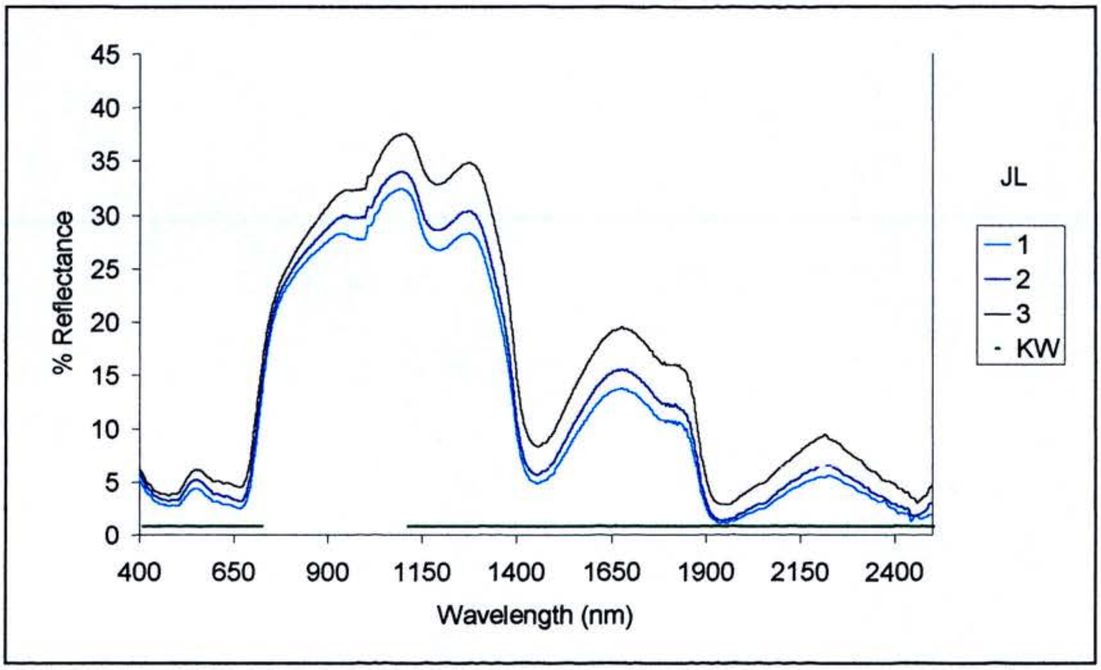

Figure 5.42. Average reflectance from *Fr* Jupiter (NT) low zinc treatments over time. Statistical difference between the dates reflectance is also shown ("KW").

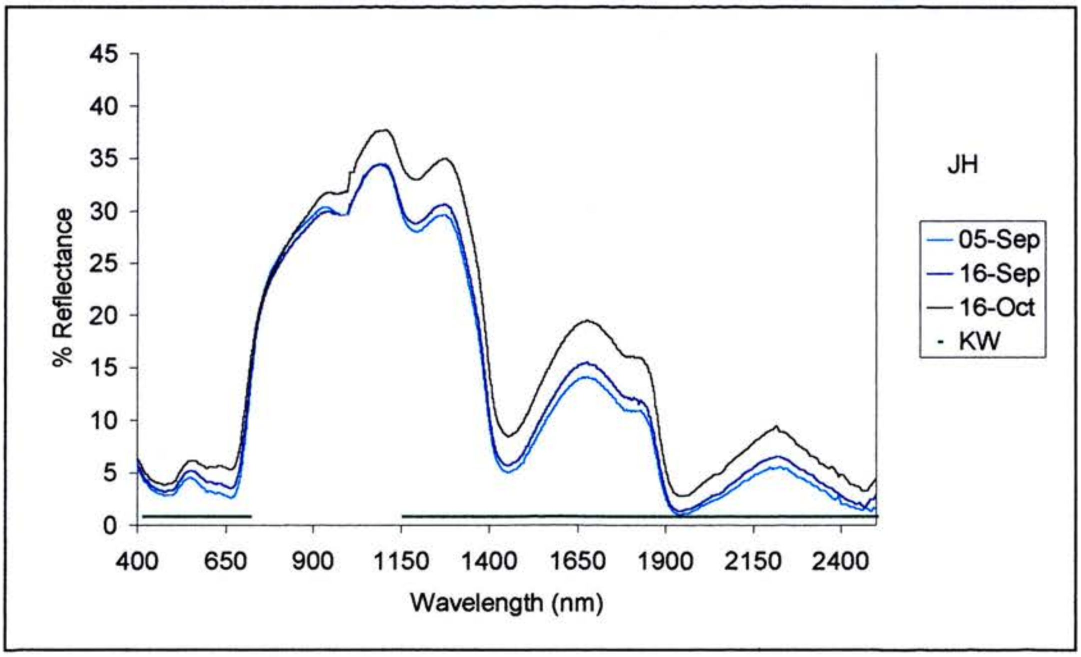

Figure 5.43. Average reflectance for *Fr* Jupiter (NT) high zinc treatments. Statistical difference between dates reflectance is also shown ("KW").

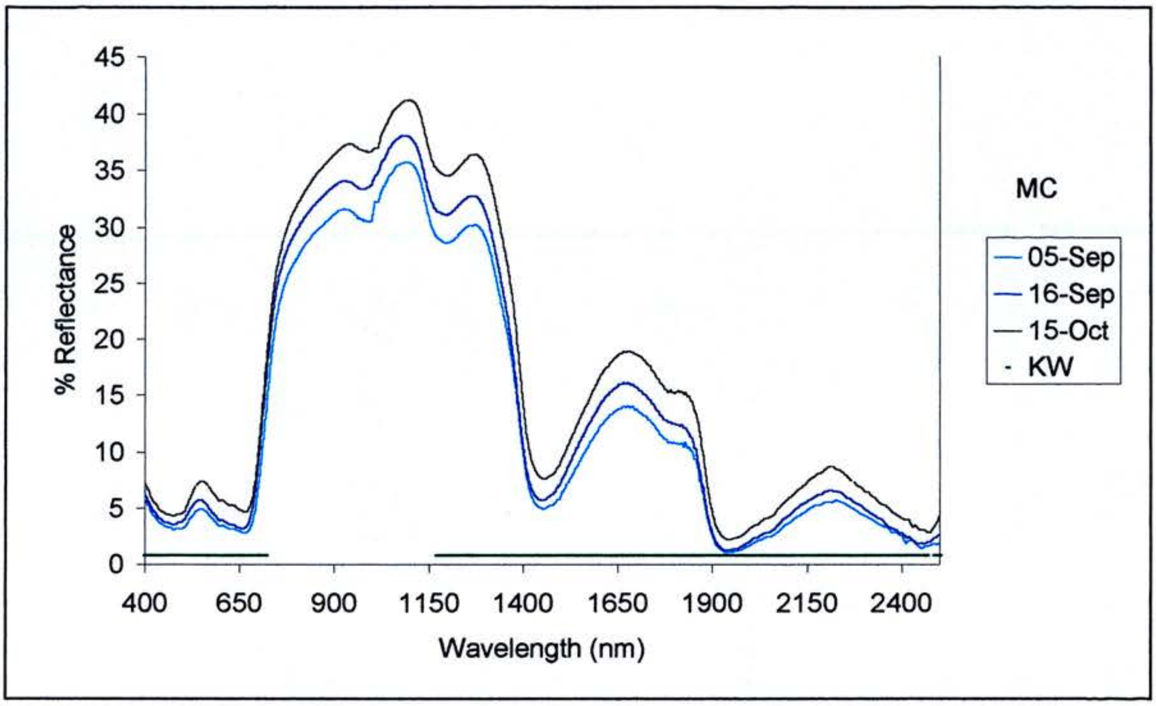

Figure 5.44. Average reflectance for *Fr* Merlin (T) control treatments over time. Statistical difference between dates reflectance is also shown ("KW").

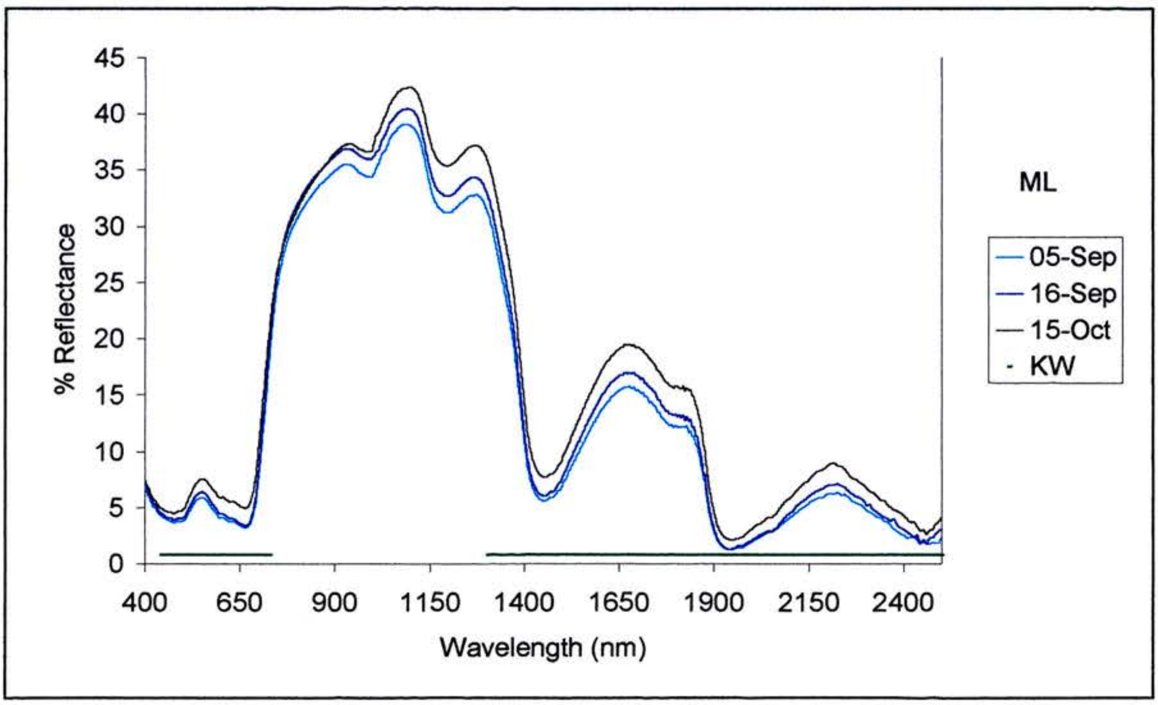

Figure 5.45. Average reflectance for *Fr* Merlin (T) low zinc treatments over time. Statistical difference between dates reflectance is also shown ("KW").

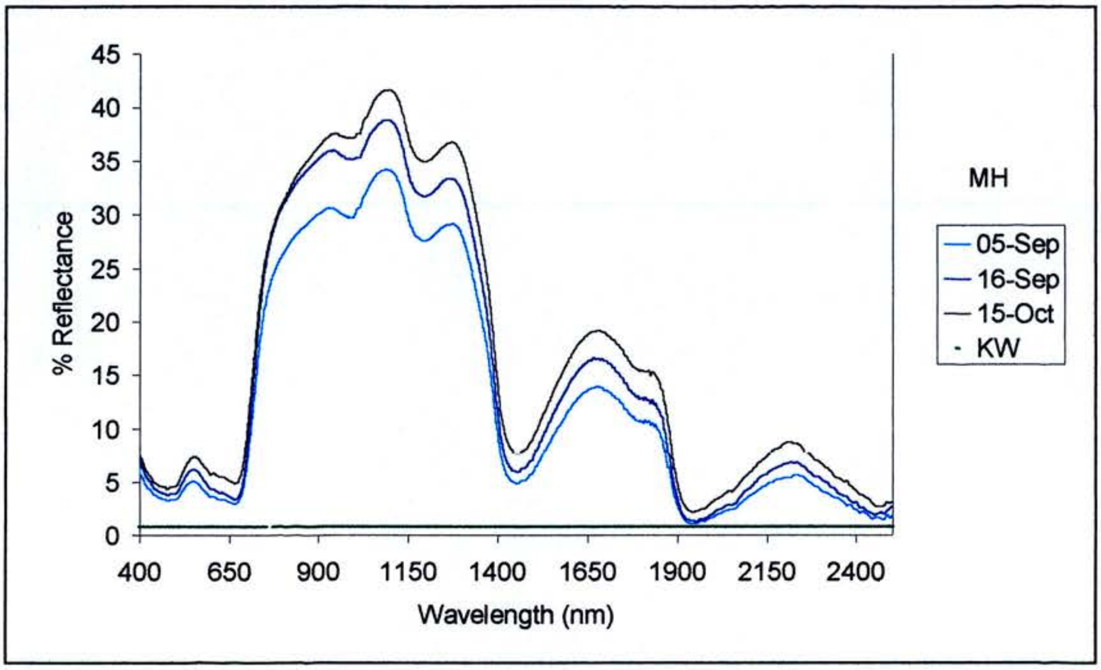

Figure 5.46. Average reflectance for *Fr* Merlin (T) high zinc treatments over time. Statistical difference between dates reflectance is also shown ("KW").

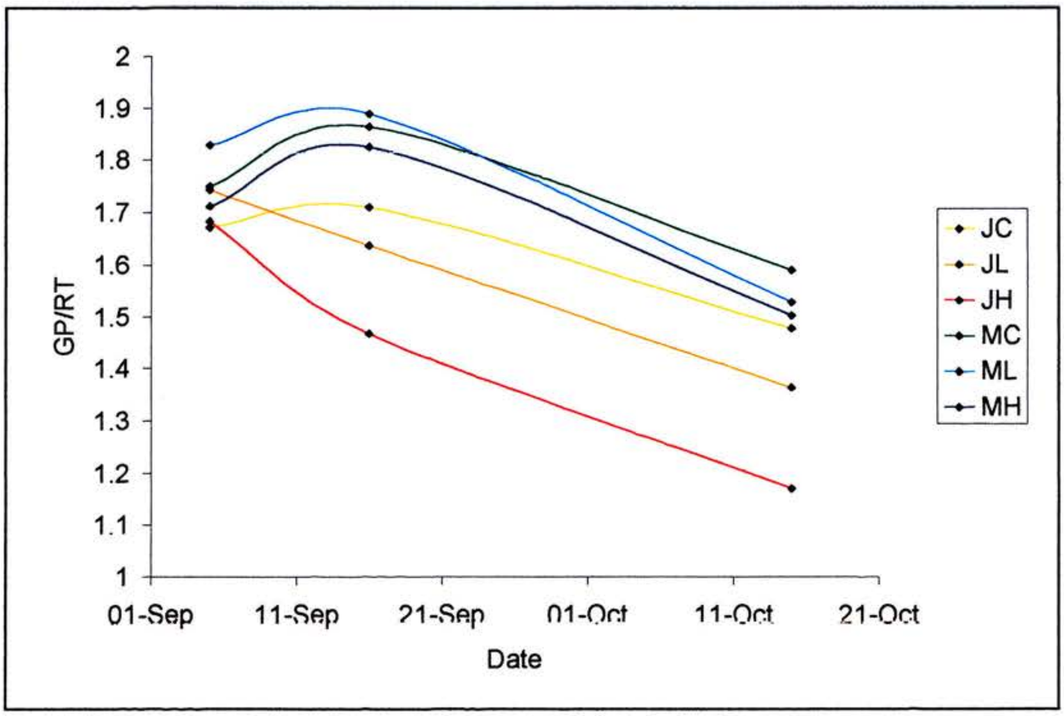

Figure 5.47. Change in GP/RT index values over time. "J" = *Fr* Jupiter (NT) treatments, "M" = *Fr* Merlin (T) treatments, "C" = control, "L" = low zinc, "H" = high zinc.

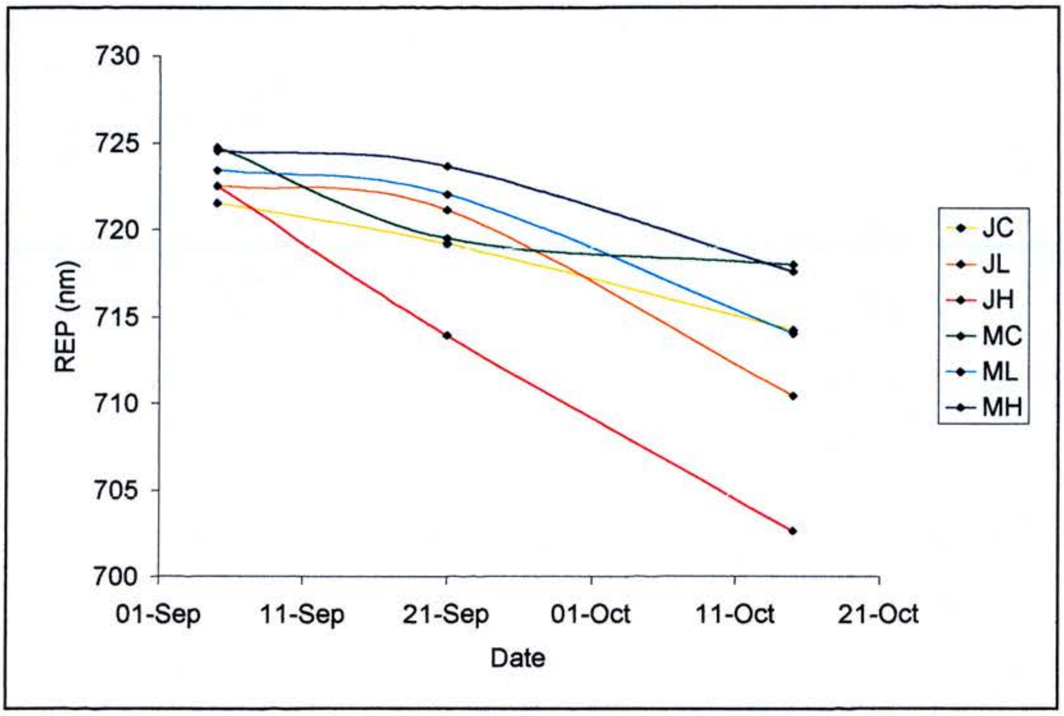

Figure 5.48. Change in average red edge position over time for all treatments. "J" = *Fr* Jupiter (NT) treatments, "M" = *Fr* Merlin (T) treatments, "C" = control, "L" = low zinc, "H" = high zinc.

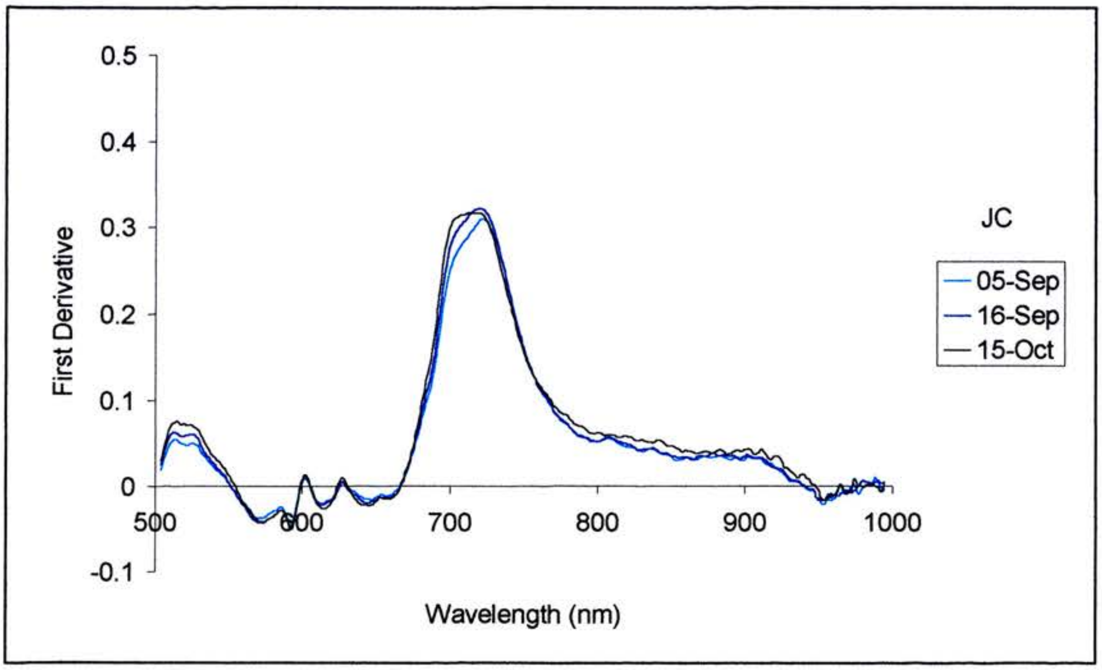

Figure 5.49. First derivative of *Fr* Jupiter (NT) control treatments over time.

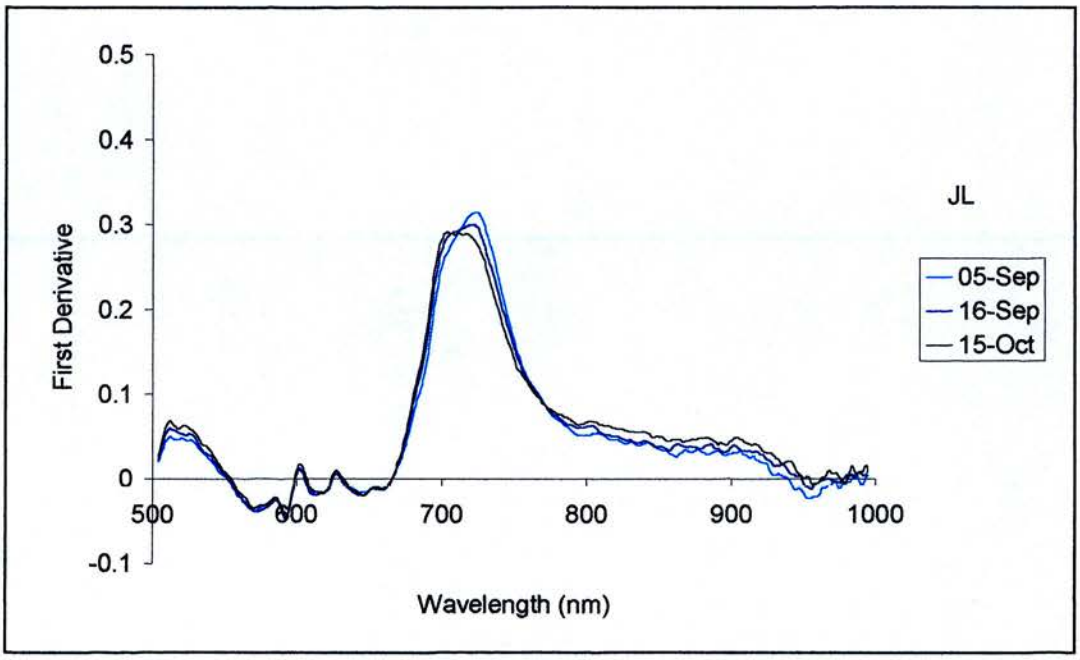

Figure 5.50. First derivative of *Fr* Jupiter (NT) low zinc treatments over time.

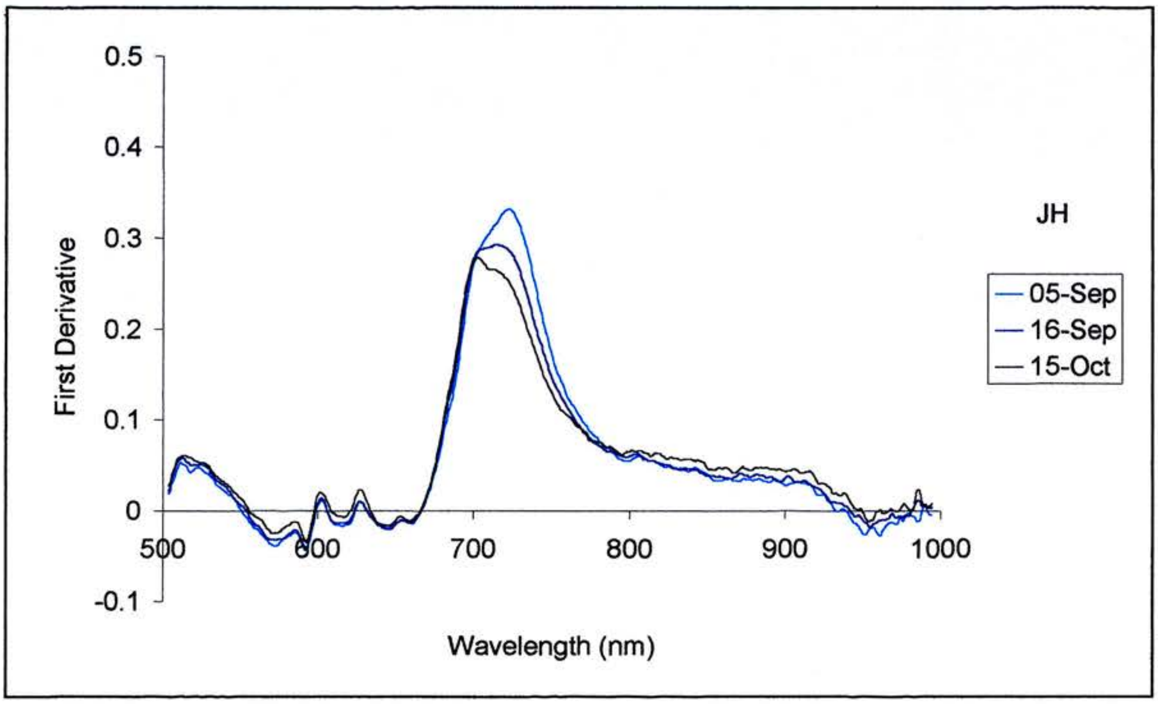

Figure 5.51. First derivative of *Fr* Jupiter (NT) high zinc treatments over time.

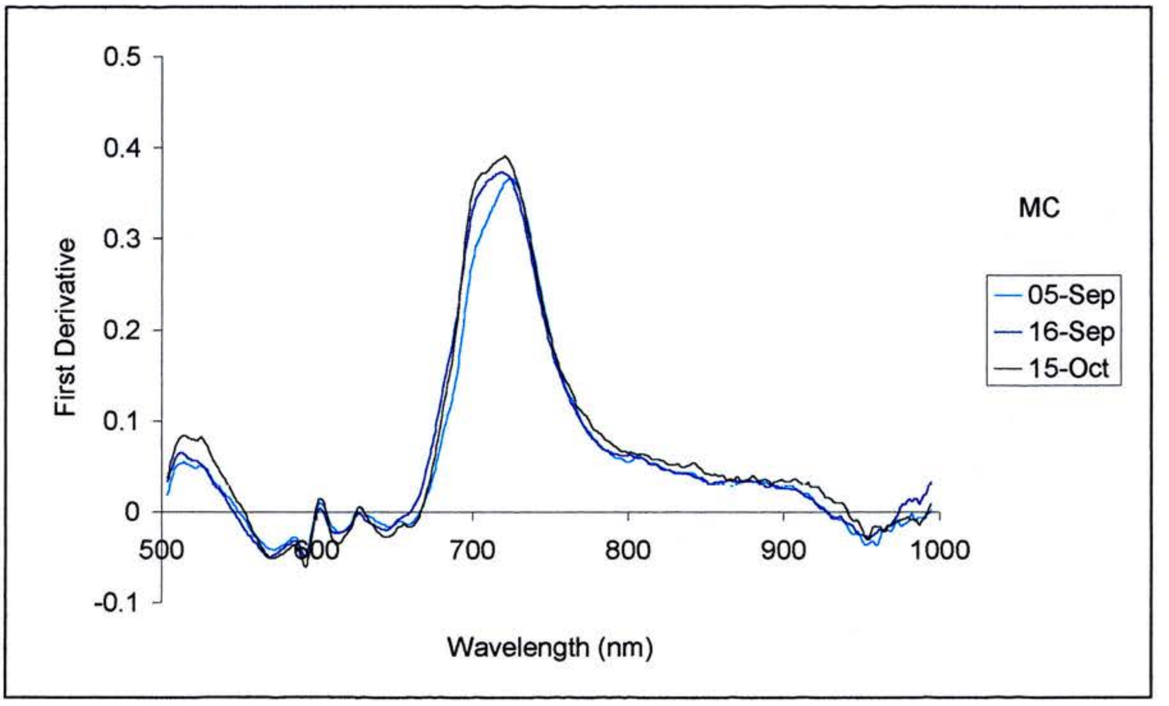

Figure 5.52. First derivative of *Fr* Merlin (T) control treatments over time.

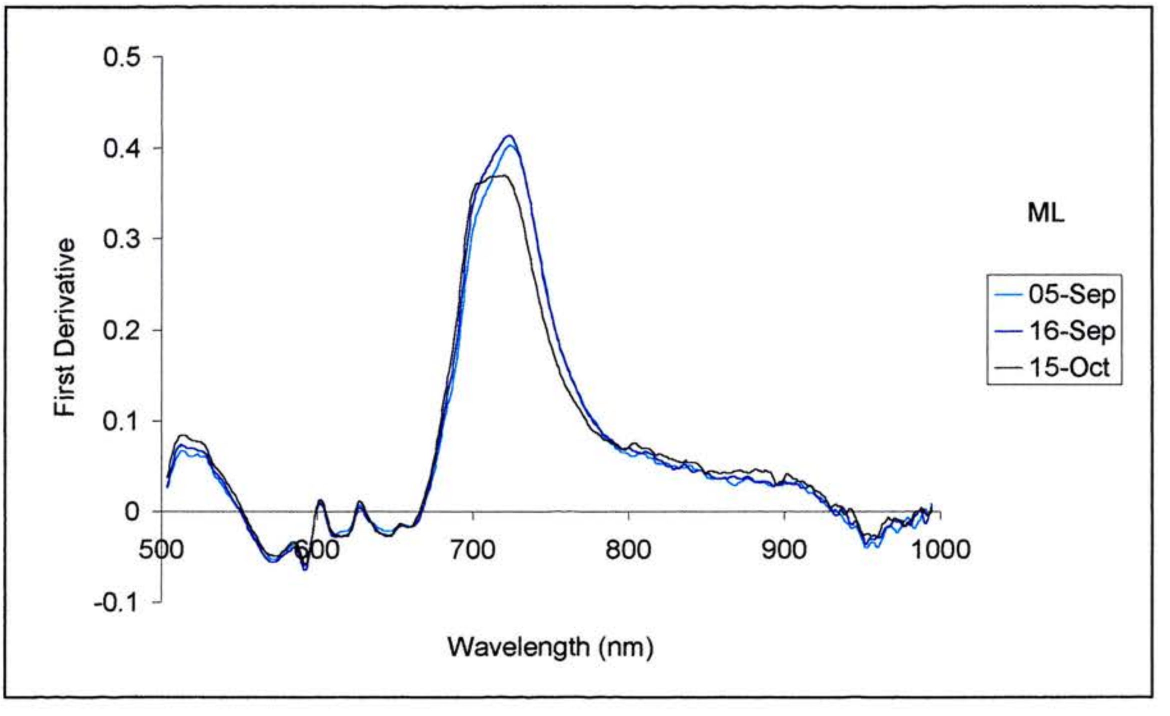

Figure 5.53. First derivative of *Fr* Merlin (T) low zinc treatments over time.

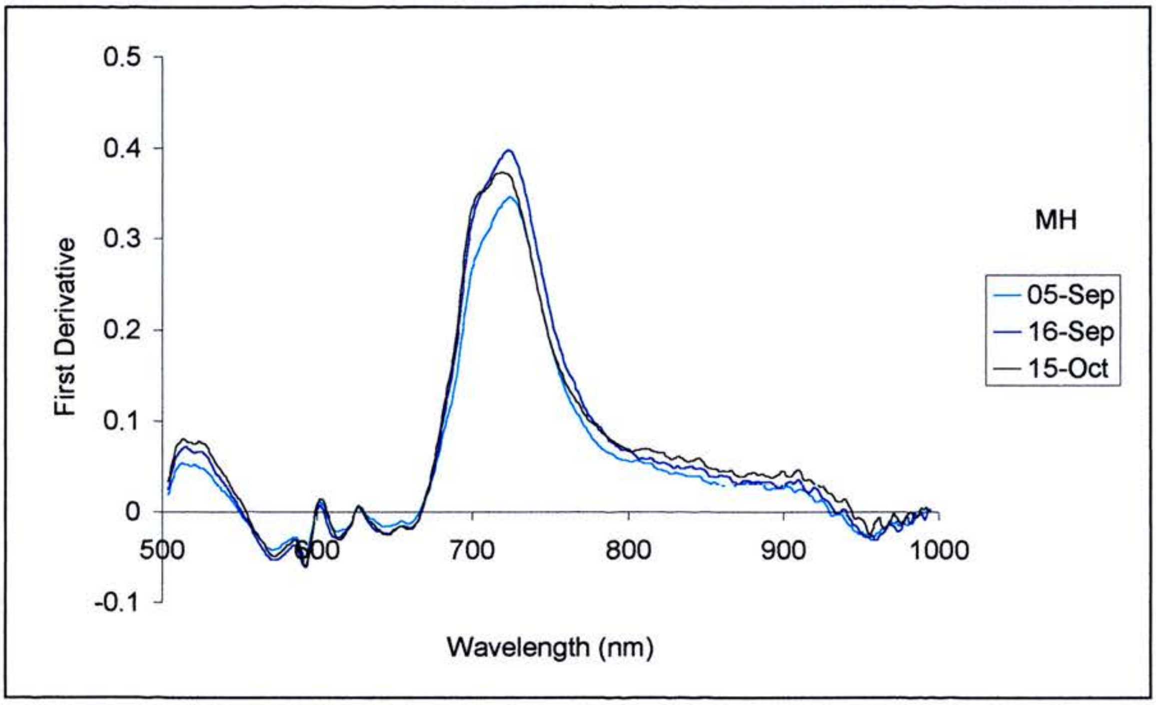

Figure 5.54. First derivative of *Fr* Merlin (T) high zinc treatments over time.

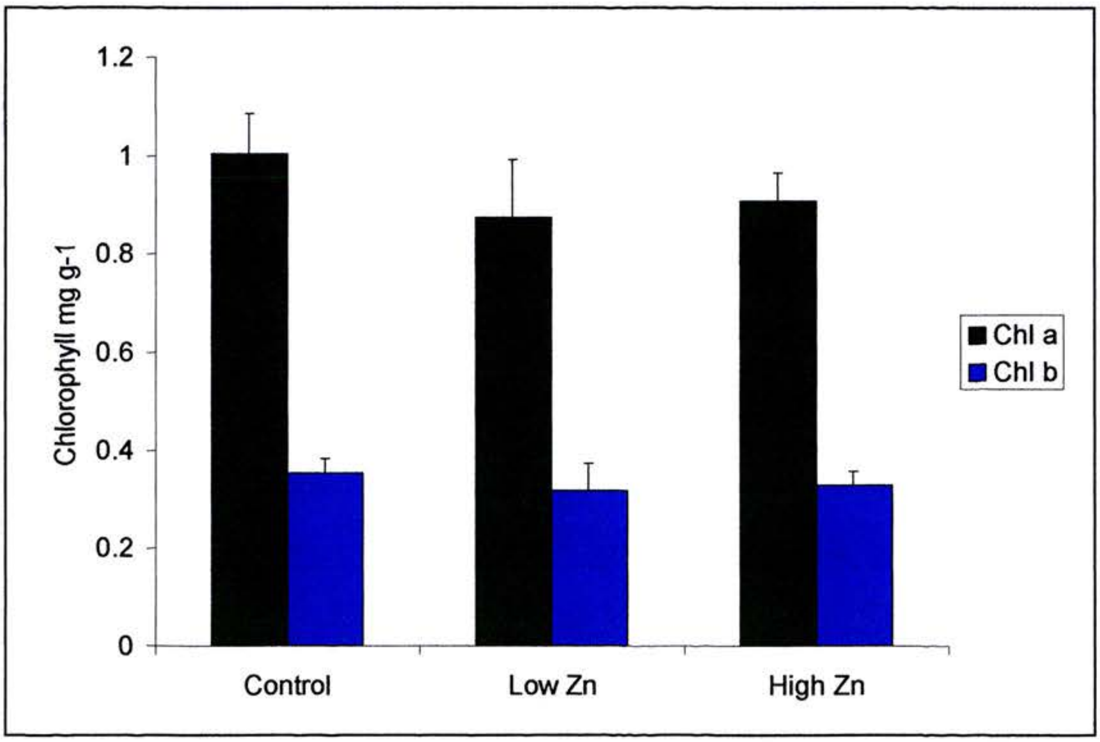

Figure 5.55. Chlorophyll results Oct. 22nd. Error bars show +1 St.Dev.

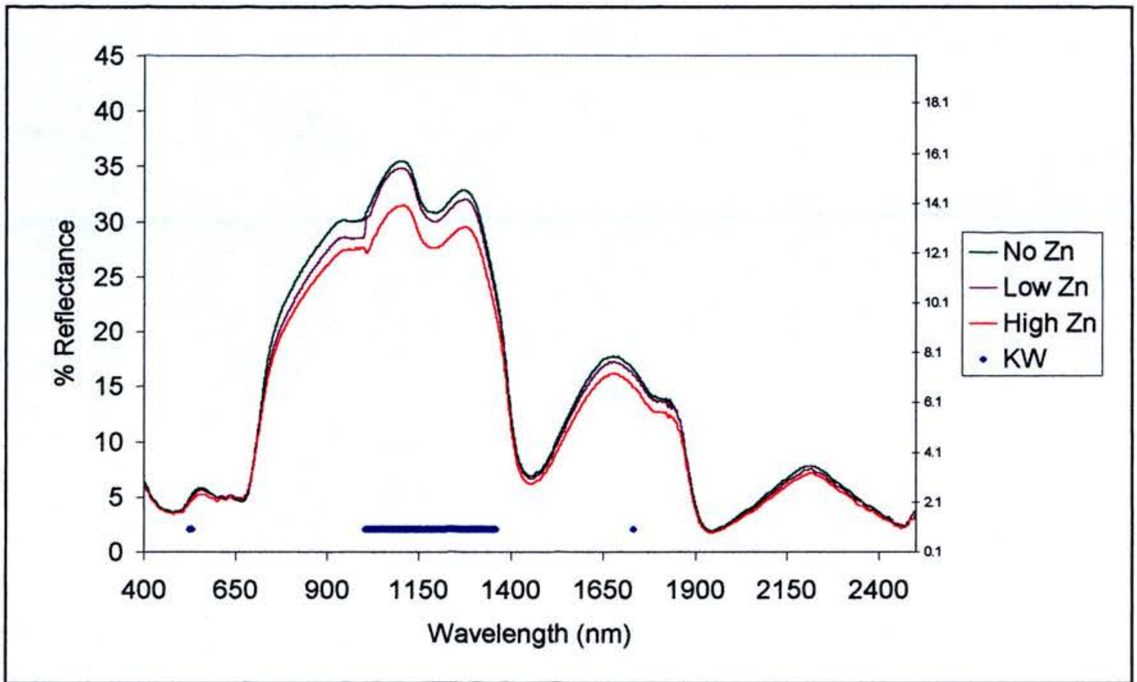

Figure 5.56. Average treatment reflectance for all mixture treatments, Oct 22nd. Statistical differences between treatments is also shown ("KW").

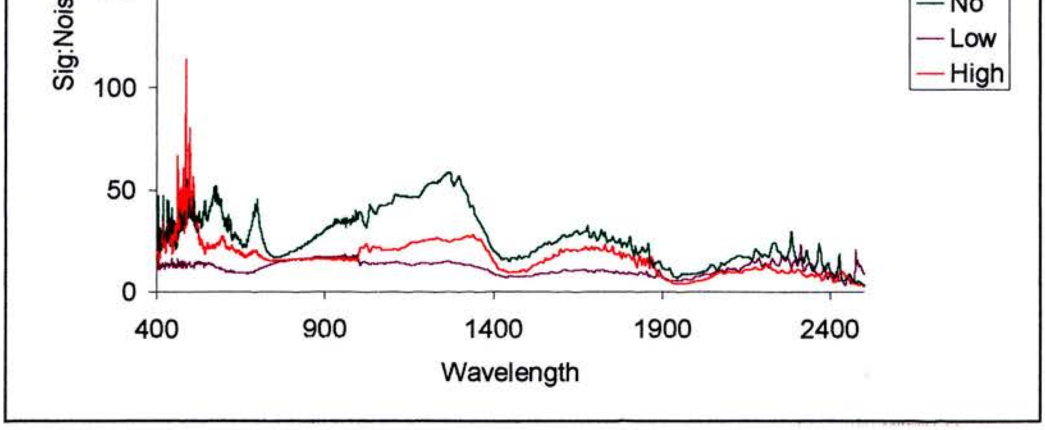

Figure 5.57 Signal : Noise relationships for all treatments, Oct. 22nd.

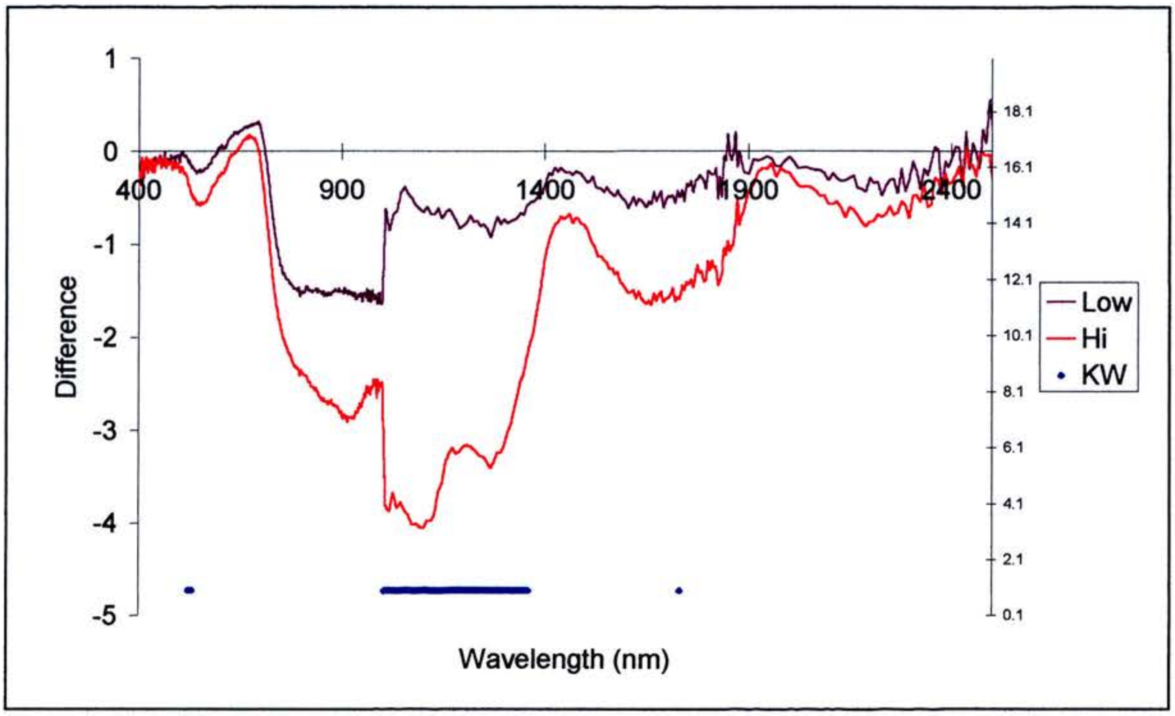

Figure 5.58. Reflective difference results for the low and high zinc treatments relative to the control, Oct. 22nd. Statistical differences between treatments are also shown ("KW")

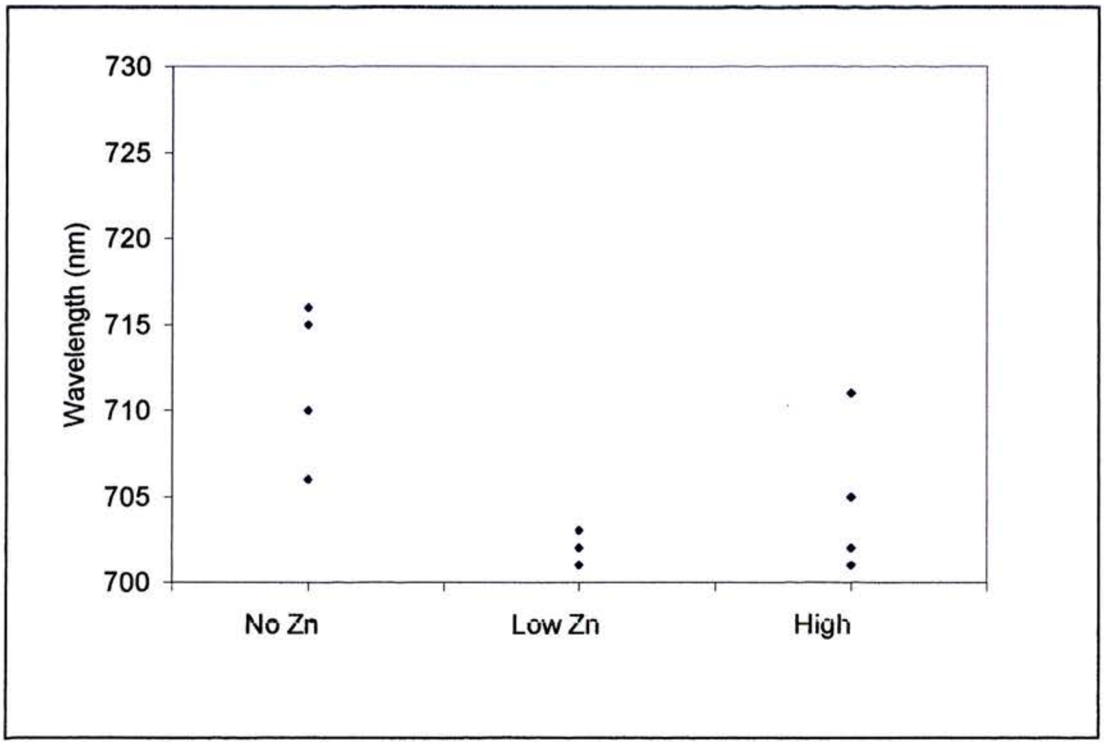

Figure 5.59. Red edge position for all treatments, Oct 22

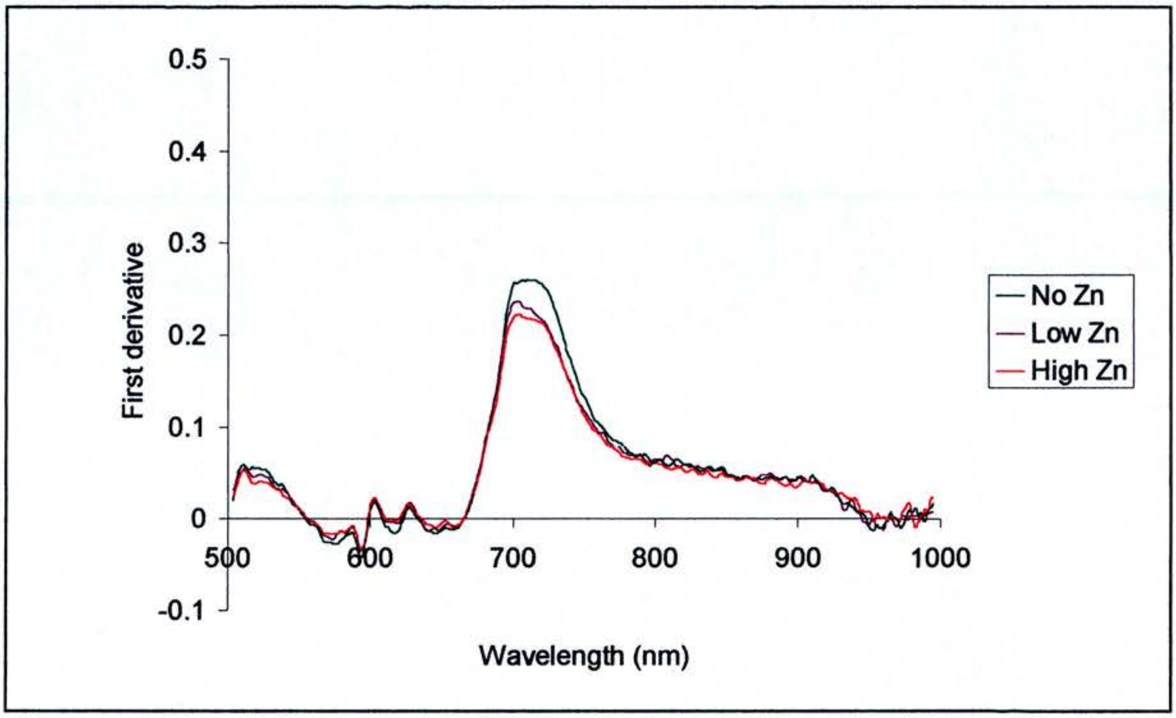

Figure 5.60. First derivative results for all treatments, Oct 22nd.

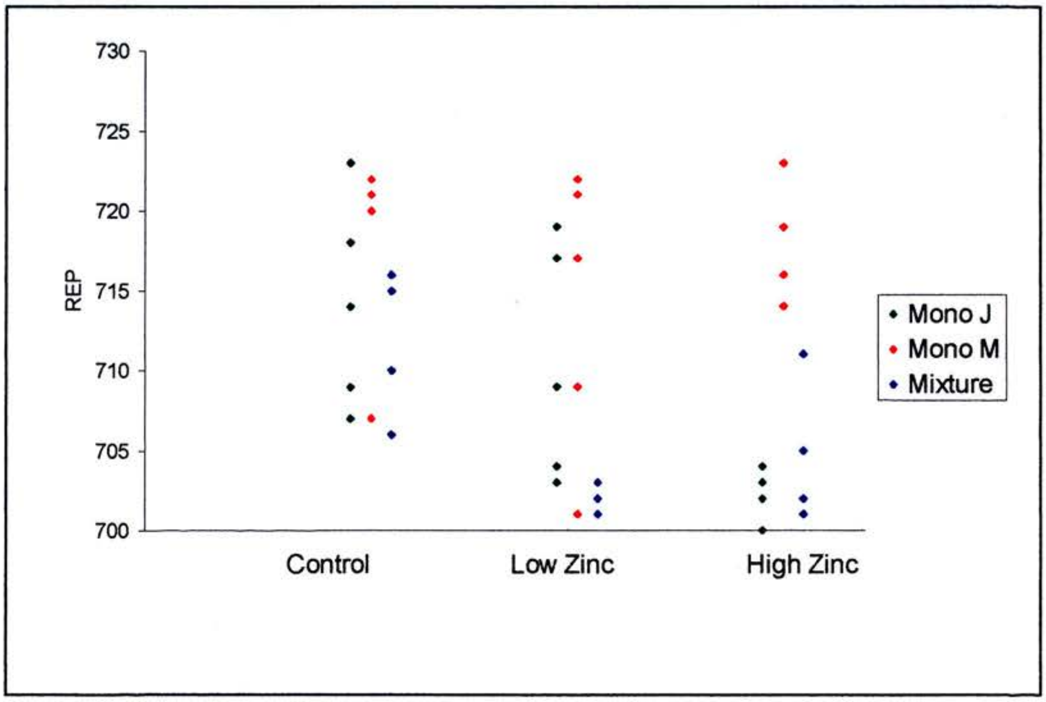

Figure 5.61 Red edge position for monoculture results on Oct 5th 2000 ("Mono J" = *Fr* Jupiter (NT); "Mono M" = *Fr* Merlin (T)), and mixture results from Oct 22nd 1999 ("Mixture"), at control, low and high zinc treatments.

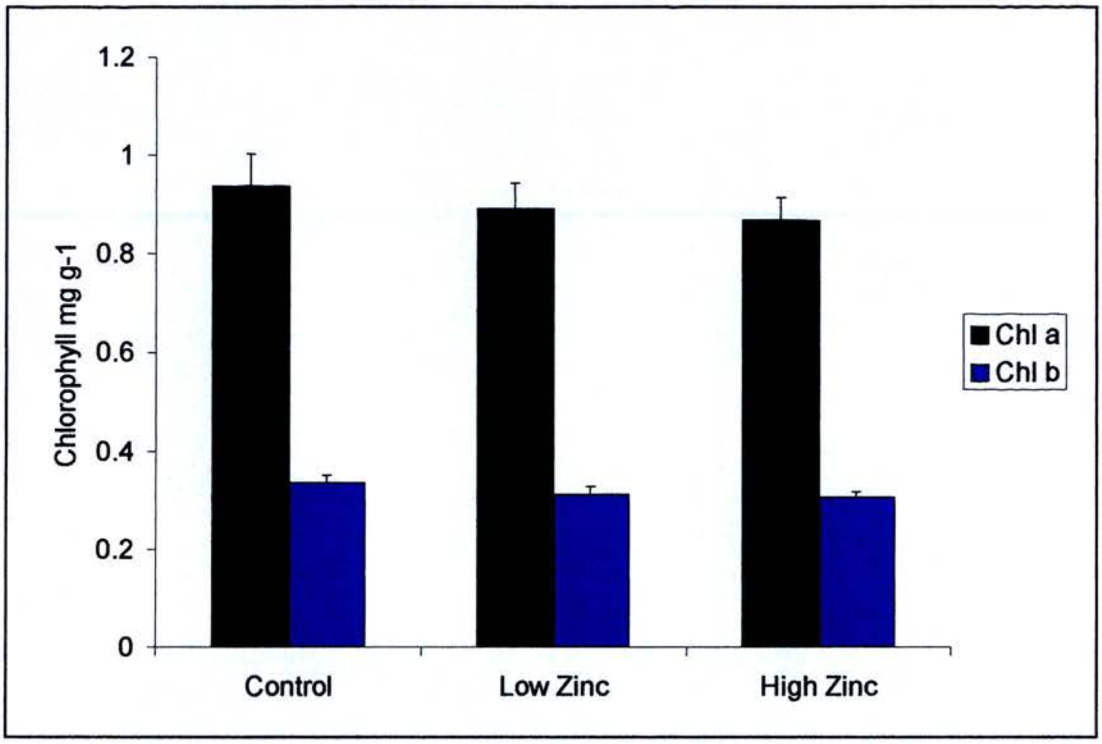

Figure 5.62. Chlorophyll results 16 Nov. Error bars show +1 St.Dev.

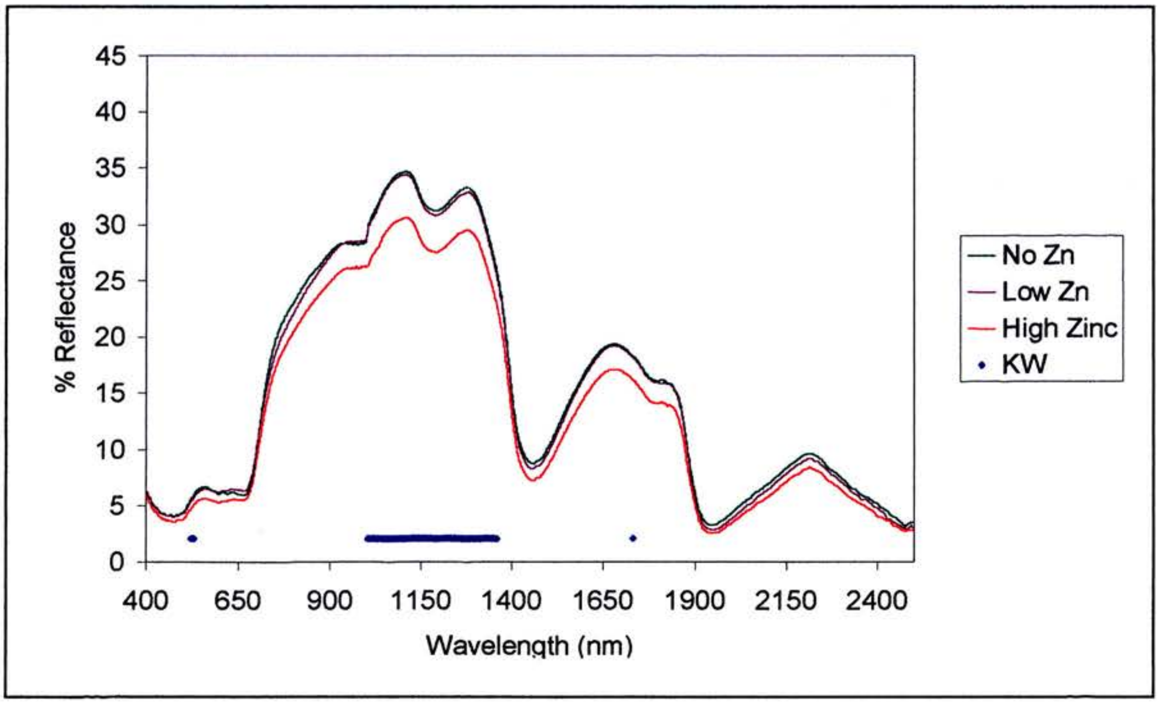

Figure 5.63. Average treatment reflectance for all treatments and associated statistical test ("KW") for Nov 16th.

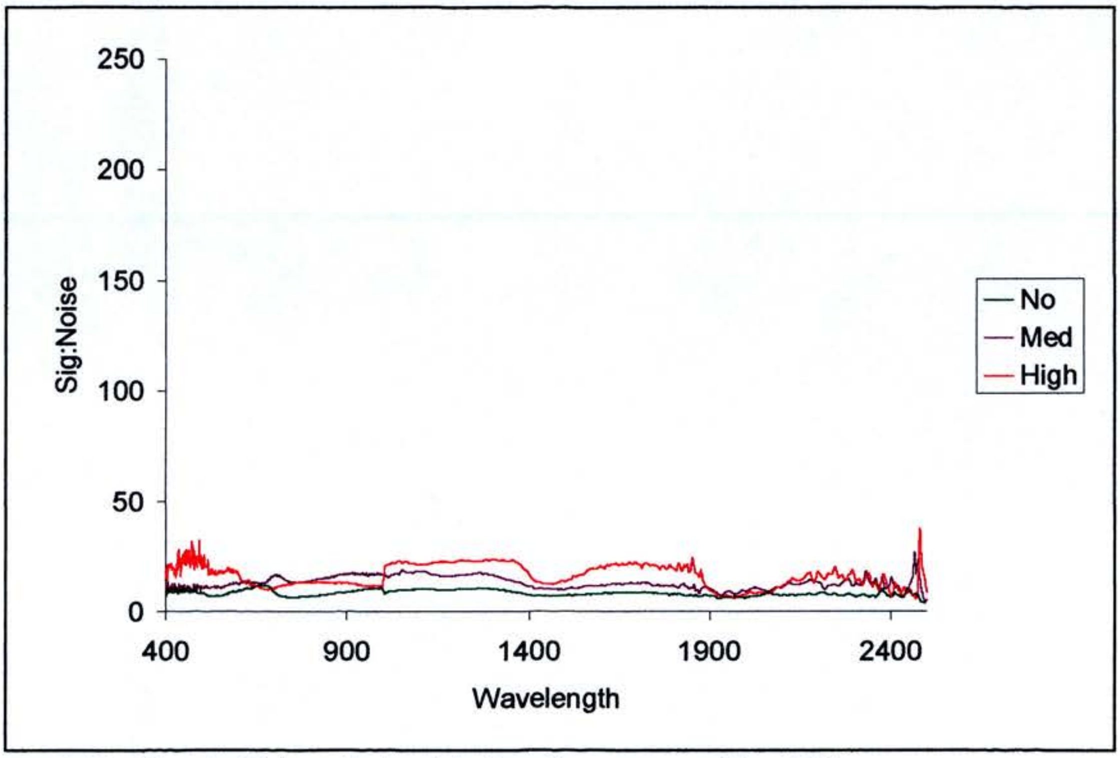

Figure 5.64 Signal : Noise relationship for all treatments, Nov 16th.

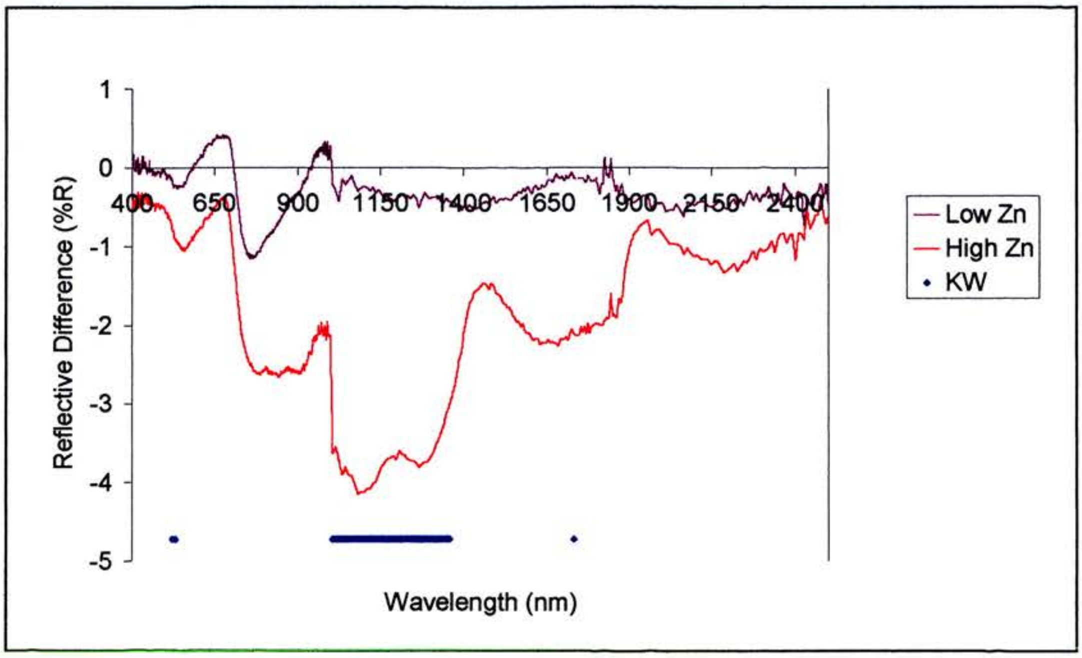

Figure 5.65. Reflective difference for all treatments, Nov 16th.

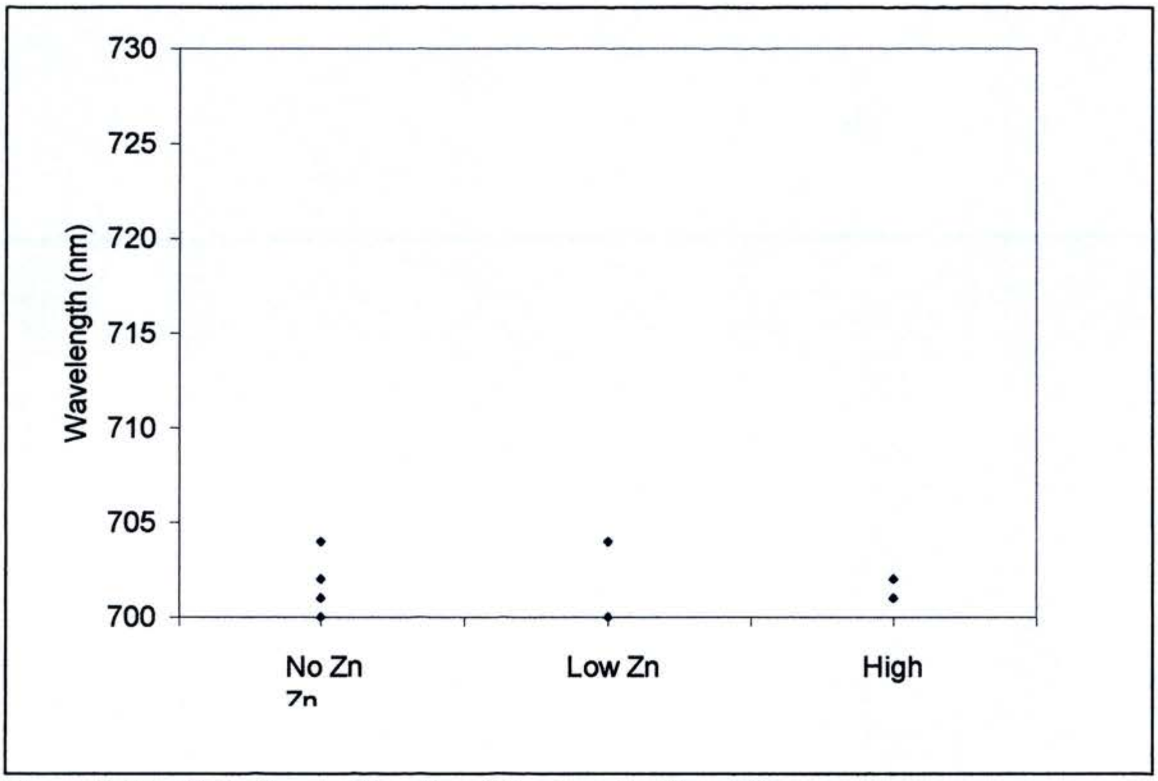

Figure 5.66. Red edge position for all treatments, Nov 16th.

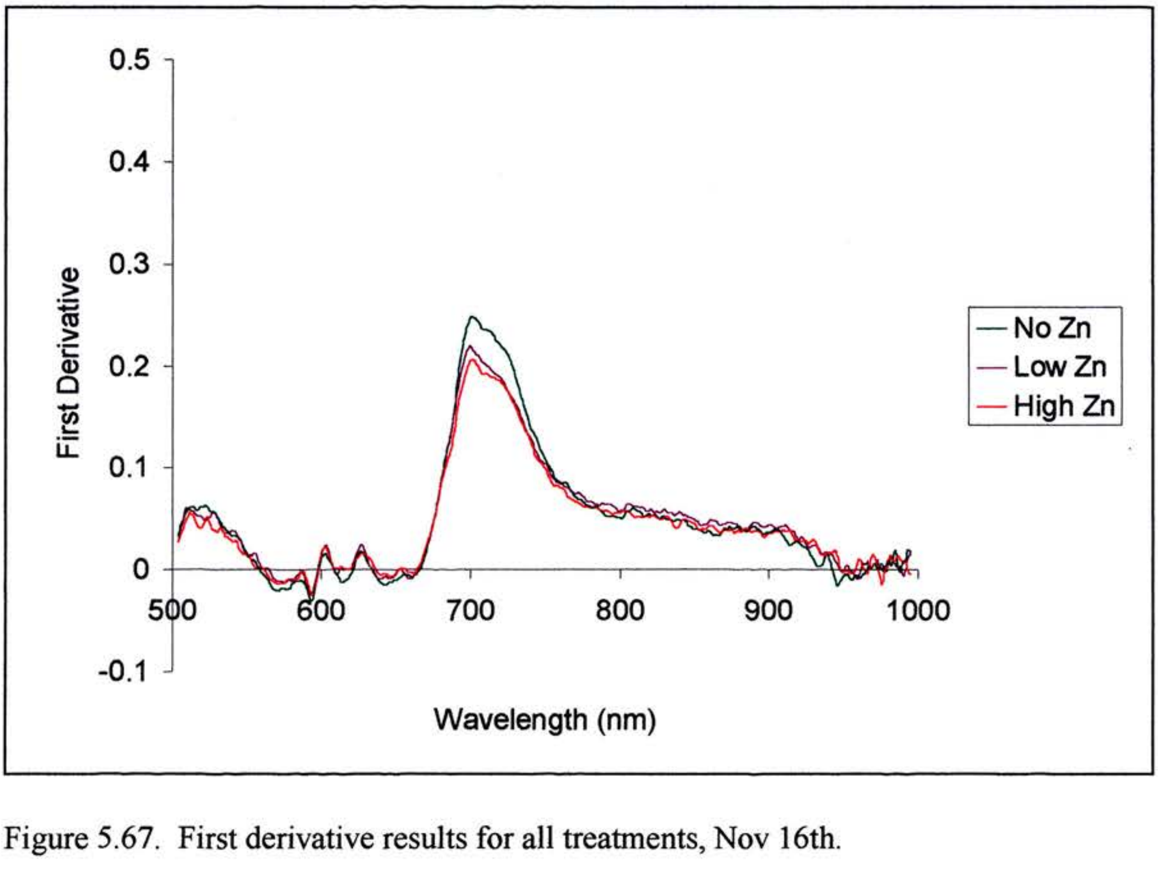

Figure 5.67. First derivative results for all treatments, Nov 16th.

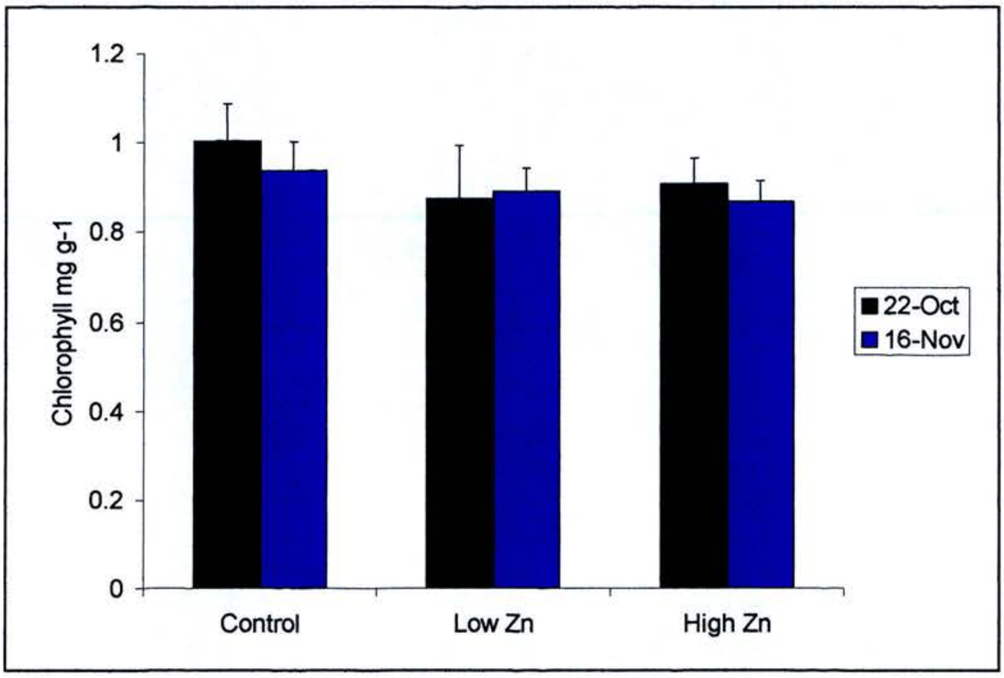

Figure 5.68 Chlorophyll results over time for the mixed canopy data. Error bars show +1 St.Dev.

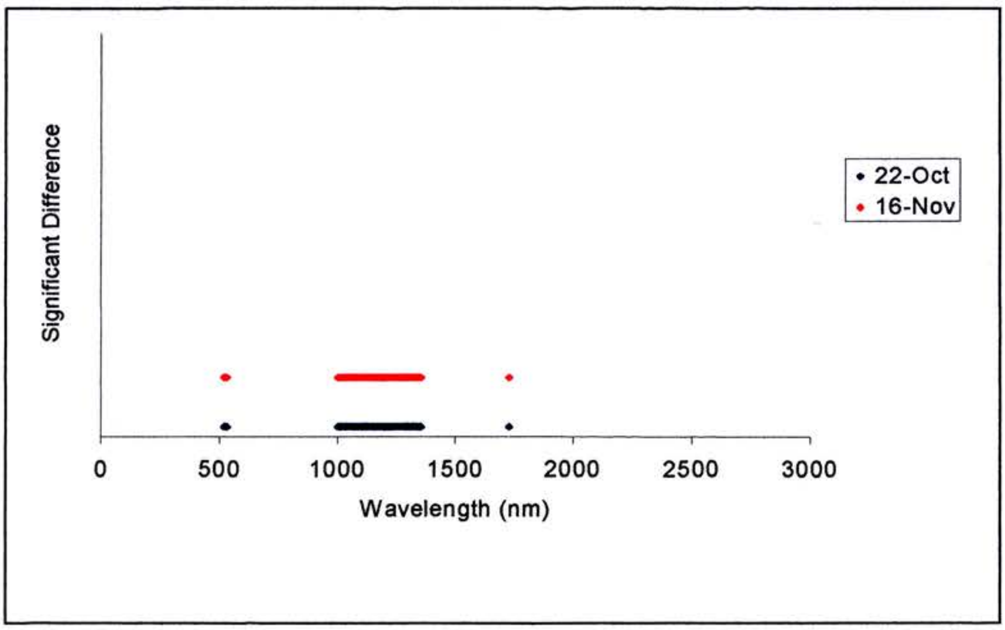

Figure 5.69. Wavelengths at which there was a significant difference between the control and high treatments on both dates for the mixed canopy data.

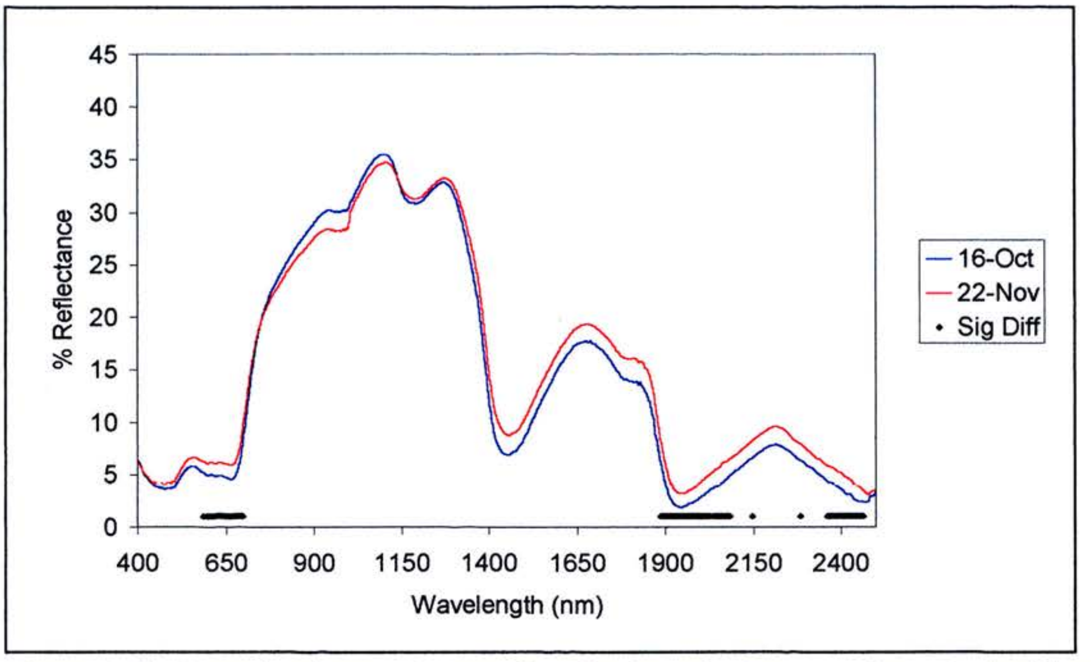

Figure 5.70 Control reflectance's over time for the mixed canopy data. A significant difference is indicated as "Sig Diff".

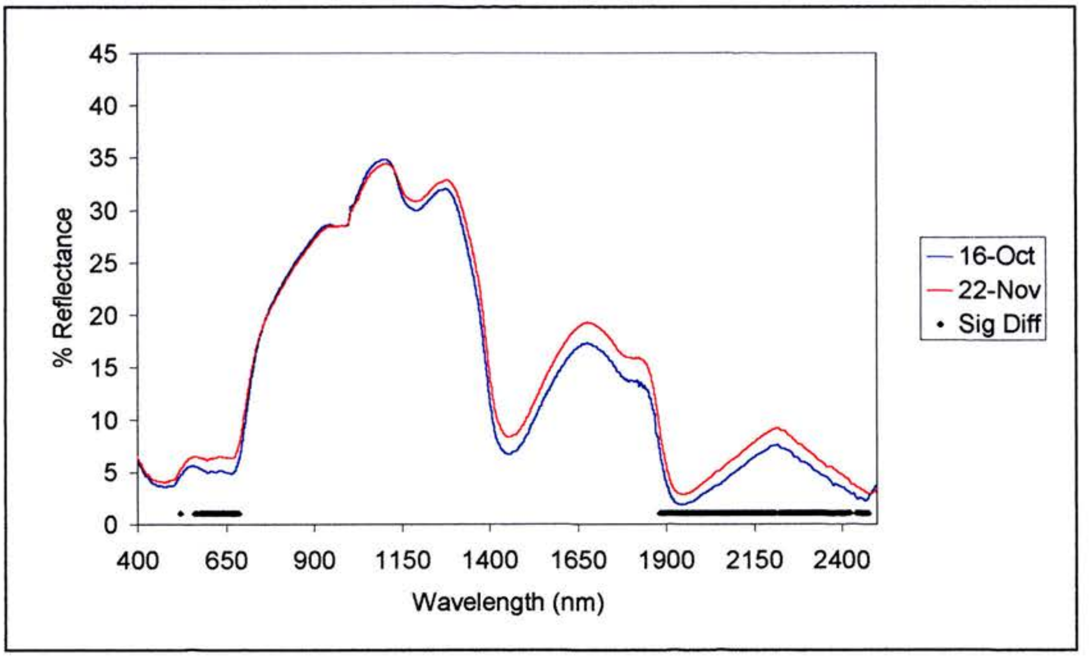

Figure 5.71 Low zinc treatment reflectance over time for the mixed canopy data. A significant difference is indicated as "Sig Diff".

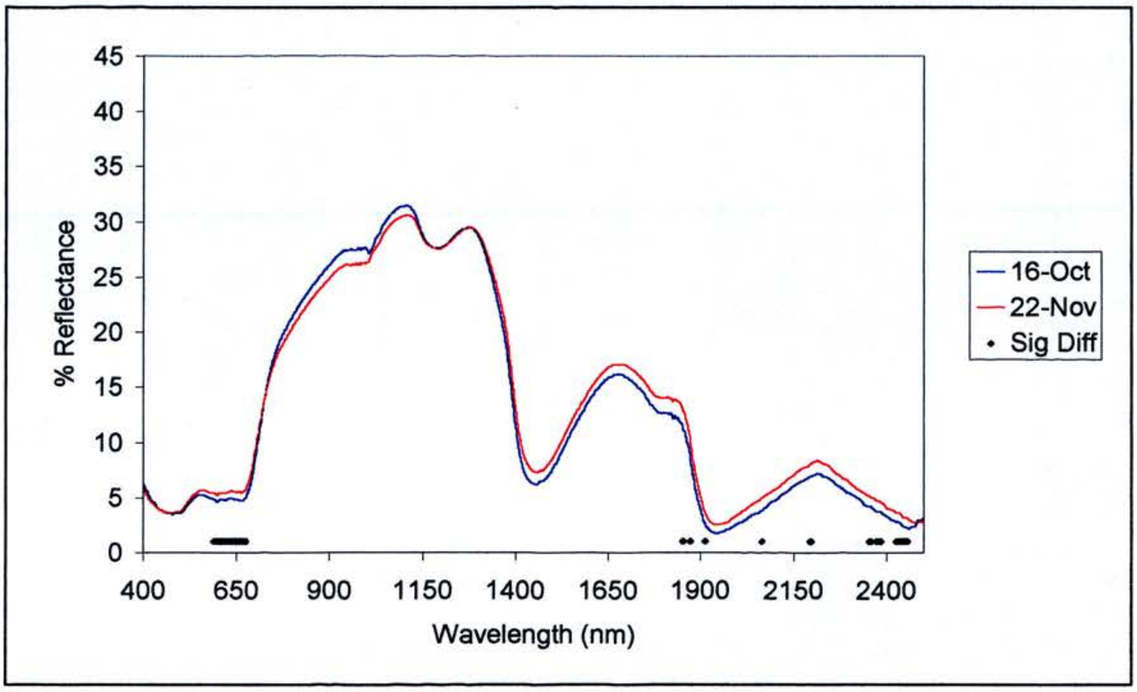

Figure 5.72 High treatment reflectance's over time for the mixed canopy data. A significant difference is indicated as "Sig Diff".

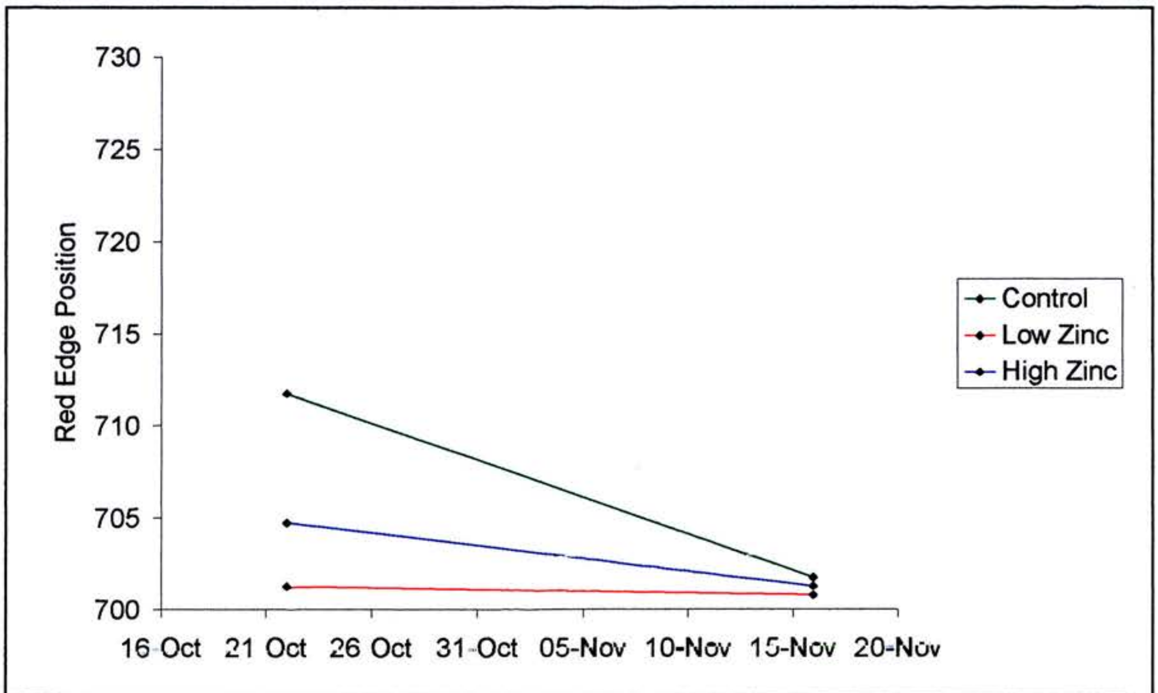

Figure 5.73. Change in red edge position over time for each treatment in the mixed canopy data.

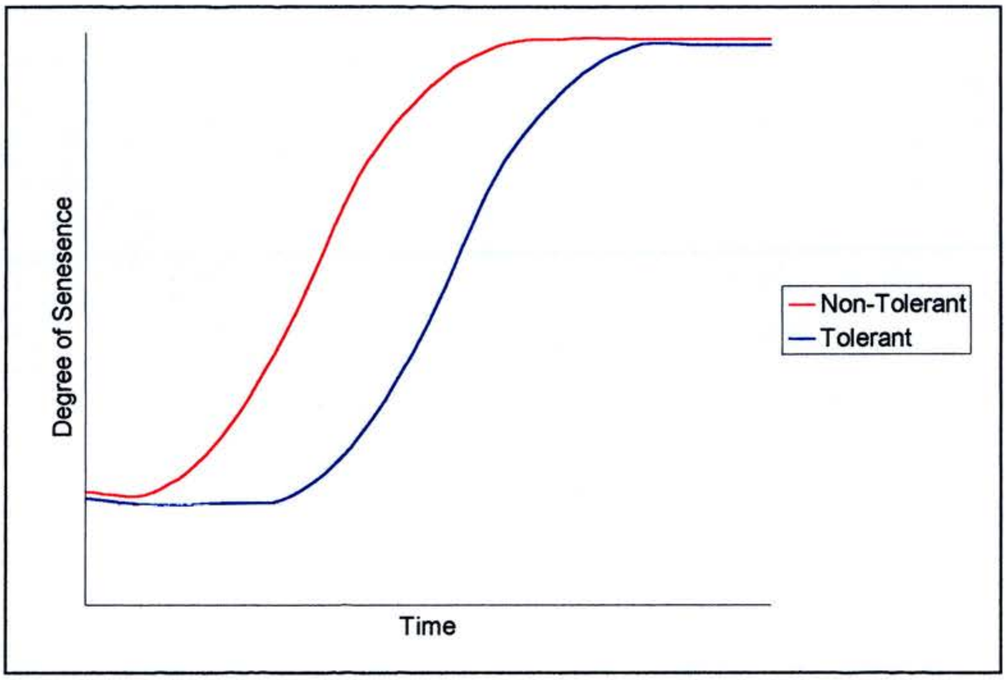

Figure 5.74. Difference in timing of senescence in stress exposed plants. The line for "Tolerant" plants is the same as the line for non-stressed non-tolerant plants. See also chapter 2, section 4.3. After Labovitz *et al.*, (1985); Schwaller and Tkach, (1985).

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Chapter 6: Modelling canopy reflectance of physiological and community responses to stress.

6.1 Introduction

Modelling provides an alternative technique to experimentation for relating reflectance characteristics to the features that caused it. Canopy radiative transfer modelling involves the simulation of reflectance from given plant characteristics (e.g. chlorophyll content, leaf area index, leaf angle). The aim in creating an accurate model of reflectance is to relate reflectance to the physical parameters that cause that reflectance through model inversion (Goel, 1988). In the development of models there is a compromise between accuracy of the model (required to be high) and the number of parameters (required to be low to ease inversion). A larger number of parameters is required to increase accuracy, so the difficulty of inversion increases (Danson *et al.*, 1995; Goel, 1988). Inversion is generally only successful if some parameters are predetermined (Plummer, 2000). Modelling also assumes that there are unique variables for any given reflectance, probably an oversimplification. Given the difficulty of inversion, modelling is included here only as a way of extending the study to the possible different community compositions introduced in Chapter 2 that are difficult to reproduce experimentally given the time available, not as a technique for obtaining canopy or leaf characteristics from reflectance data..

PROSPECT (Jacquemoud and Baret, 1990; Jacquemoud *et al.*, 1996) (a radiative transfer model) was used to model leaf reflectance. It is an improved version of the Allen *et al.* (1969) plate model (cited in (Jacquemoud and Baret, 1990)). It has four input parameters, N (corresponding to leaf structure), C_{ab} (total chlorophyll content), C_w (water content), and C_m (corresponding to protein/cellulose/lignin). N affects reflectance across the spectrum, although it's main effects are in the near infra-red region. PROSPECT assumes a leaf is comprised of N layers, with N-1 air spaces between layers of infinitesimal depth (N need not be an integer). The first layer gets an incident beam of light with a defined angle, the rest of the layers get diffused light. N is therefore a measure of the degree of refraction in a leaf. Monocotyledonous plants, with compact mesophyll, theoretically have a lower N (1 - 1.5) value than dicotyledons (1.5 - 2.5) which have more internal air spaces (Jacquemoud and Baret, 1990), although this distinction was not found by (Jacquemoud *et al.*, 1996). Senescent plants are represented as having a more disorganised internal structure, with higher N values. Absorption is modelled by chlorophyll content affecting visible reflectance, water affecting the middle infra red region, and protein/cellulose/lignin affecting near infra red-reflectance. Typical parameters for these inputs are given in Table 6.01. The output from the model is leaf reflectance from 400-2500 nm.

Canopy reflectance was modelled using SAIL (Scattering by Arbitrarily Inclined Leaves) (Verhoef, 1984). This is a turbid medium model (using Goel (1988) categories), which models homogeneous canopies consisting solely of leaves randomly distributed in horizontal layers. SAIL does not consider any other canopy components, which are not present in grass canopies anyway. The canopy BRDF is modelled considering leaf and soil optical properties, leaf area index (LAI), leaf angle distribution (LAD), view and solar angle. Leaf and soil reflectance are chosen depending on the particular canopy being modelled (Table 6.02). LAI, LAD, view and solar angle modify the reflectance to account for the influence of soil and canopy architecture on the canopy BRDF.

A review of plant community models found none suitable for this study. To be suitable a model had to model the response of a plant community comprised of stress tolerant and non-tolerant individuals. The outputs of the model had to be frequency of plant and soil cover along with physiological information suitable for reflectance model inputs. No models considered the tolerance of plants, and no models were designed as a counterpart to reflectance models, so the outputs were unsuitable. It would have been beyond the reach of this project to design such a model. Model input values were therefore based upon the understanding of plant response to metal inputs established in Chapters 2 and 3.

Table 6.01. Input parameters and typical values for the PROSPECT (Jacquemoud and Baret, 1990), (Jacquemoud *et al.*, 1996)model of leaf reflectance.

Parameter	Value	Notes	Source
N	1 to 1.5	For Monocotyledens	(Jacquemoud and Baret, 1990
	1.09	For Corn	(Kuusk, 1994)
	1 to 2	"Realistic range"	(Verhoef, 2000)
	1.5	"Realistic value"	(Jacquemoud *et al.*, 2000)
Chl.	15 to 80	"Realistic range"	(Verhoef, 2000)
	35	"Realistic value"	(Jacquemoud *et al.*, 2000)
C_w	0.026	For Corn	(Kuusk, 1994)
	0.015	"Realistic value"	(Jacquemoud *et al.*, 2000)
C_m	0.01	"Realistic value"	(Jacquemoud *et al.*, 2000)

NB. "Realistic Range" refers to values used by those authors investigating responses of the model to values they might expect to see in the natural environment.

Table 6.02. Input values for the SAIL (Verhoef, 1984) model of canopy BRDF.

Parameter	Value	Notes	Source
Leaf reflectance	varies	Chosen by user depending on focus of study	
Soil reflectance	varies	Chosen by user depending on focus of study	
LAI	2	"Standard canopy"	(Bacour and Jacquemoud,
LAD	63.24	Healthy erectophile canopy	(Kimes, 1984) cited in (Goel, 1988)
Illumination Angle	Any	Chosen by user depending on focus of study	
View Angle	Any	Chosen by user depending on focus of study	

6.2 Method.

The canopy reflectance of simple communities was modelled to simulate the effects of stress and changes in community structure in response to different levels of metal contamination. The community modelled consisted of non-tolerant and tolerant ecotypes of a species. Their physiology and relative community composition was modelled. Canopy reflectance for each ecotype was modelled in response to the imposition of stress. The community composition was then modelled with non-tolerant, tolerant and bare soil being assigned a percentage cover. Simple mixture modelling was used to derive community reflectance from modelled plant canopy and soil reflectance. The different components (non-tolerant, tolerant and soil) were considered independent and were added after being weighted by their respective cover fractions.

6.2.1 Modelling plant reflectance

Each ecotype had its reflectance modelled using a combined PROSPECT-SAIL (PROSAIL) model provided by (Danson, 2001). The model was run on MATLAB version 5.3 (Student Edition). PROSPECT inputs were modelled and inputted into SAIL which modelled canopy reflectance (1 nm intervals) based on leaf and soil reflectance, canopy architecture view and solar angle. Soil reflectance from a peat/sand mix was used (provided by Dr T Malthus, Figure 6.01)). In the version of model used in this study view zenith and solar azimuth are set to 0, and solar zenith angle is set to 45 degrees, with diffuse skylight set to 0.2 (Danson, 2001). This is adequate because the main interest is in canopy stress and community composition effects on reflectance, not BRDF. The remaining variables were altered to represent the different ecotypes under different levels of stress.

The physiological response of non-tolerant plants was modelled in response to high soil metal contents (Table 6.03). The control values are based on typical monocotyledon values (Table 6.01, 6.02). With stress LAI decreases (Knipling, 1970), N increases (Jacquemoud and Baret, 1990), chlorophyll decreases (Boochs and Kupfer, 1990), as does water content (Atkins *et al.*, 1982), and biomass (Nagy and Procotor, 1997). Erectophile leaves were assumed to become more horizontal with stress. The end values (t5) were chosen based on the extremes of ranges given by (Verhoef, 2000) and an understanding of plant physiology (Chapter 2). The t-values in between control and t5 step down in even quantities, and values for dead plants are given at t6.

Table 6.03. Values used in the PROSAIL model for the response of non-tolerant plants to high levels of metal input. Control is pre-metal, and t1...t6 correspond to time after dose.

Parameter	Control	t1	t2	t3	t4	t5	t6 (dead)
LAI	3	2.5	2	1.5	1	0.5	0.5
N	1.5	1.6	1.7	1.8	1.9	2	2
C_{ab}	60	50	40	30	20	10	0
C_w	0.05	0.041	0.032	0.023	0.014	0.005	0
C_m	0.025	0.022	0.019	0.016	0.013	0.01	0.01
LAD	65	55	45	35	35	35	35

The response to low levels of stress (e.g. for a mild dose of metal contaminant) for the non-tolerant ecotype was also modelled (Table 6.04). These values were chosen based on the same control values as it is the same ecotype, with an endpoint change in values half of that for the high stress situation at t5. e.g. LAI changes from 3 to 0.5 with high stress, so for the low stress it changes from 3 to 1.75 in equal steps.

Table 6.04. Values used in PROSAIL model for the response of non-tolerant plants to low levels of input. Control is pre-metal, and t1...t5 correspond to time after dose.

Parameter	Control	t1	t2	t3	t4	t5
LAI	3	2.75	2.5	2.25	2	1.75
N	1.5	1.55	1.6	1.65	1.7	1.75
C_{ab}	60	55	50	45	40	35
C_w	0.05	0.0455	0.041	0.0365	0.032	0.0275
C_m	0.025	0.0235	0.022	0.0205	0.019	0.0175
LAD	65	62	59	56	53	50

The input values for tolerant plants in the control situation were the same as for the non-tolerant ecotype (Table 6.05). The tolerant plant inputs were modelled not to respond to low levels of stress, but to have a slight response to high levels of stress (Table 6.05).

Table 6.05. Values used in PROSAIL model for the response of tolerant plants to all levels of input.

	Treatment		
Parameter	Control	Low	High
LAI	3	3	2.5
N	1.5	1.5	1.6
C_{ab}	60	60	50
C_w	0.05	0.05	0.041
C_m	0.025	0.025	0.022
LAD	65	65	55

6.2.2 Modelling community composition

The community response was modelled considering the following assumptions:

- Only one species present with two ecotypes, metal tolerant and metal non-tolerant.
- Metal content is the only variable affecting model inputs.
- Without contamination non-tolerant plants are able to outcompete tolerant plants, so tolerant plants are either at very low levels or are not present.
- At low metal levels the cover of non-tolerant plants is not reduced unless they are outcompeted by the tolerant plants.
- At high metal levels the cover of non-tolerant plants is reduced.
- The tolerant ecotype can outcompete the non-tolerant ecotype if metal is present.

The output of this modelling is the proportion of non-tolerant plants, tolerant plants and bare soil at each time step. Four community responses were modelled:

- low stress with both ecotypes present
- high stress with both ecotypes present
- low stress of only non-tolerant plants followed by invasion of tolerant plants
- high stress of only non-tolerant plants followed by invasion of tolerant plants.

Where the model inputs changed with time (e.g. Table 6.04) the first 5 iterations used those values, and following iterations used the last value from the table. For example, for low contamination at time 0 (t0) the control values are used, after which t1 values then t2 through to t5, and t5 values are used for the rest of the iterations. This is to represent the gradual imposition of stress on the plants from the contamination, followed by a constant stress applied.

The community response to a low level of stress with both ecotypes present was modelled (Figure 6.05; Community 1). This community already had tolerant plants present at a low level (1%). Initially there was no change in community composition as the non-tolerant plants could still outcompete the tolerant plants. Non-tolerant plants had an increasing stress response with time which remained constant after t5 (Table 6.04). At t4 stress reduced the competitive ability of non-tolerant plants enough that tolerant plants could outcompete them. This resulted in a linear increase in the proportion of tolerant plants, and a concomittant linear decrease in proportion of non-tolerant plants. As replacement occurred there was no stage where the soil was directly visible.

The response of a community to high levels of metal input with both ecotypes present (tolerant plants at 1%) is given in Figure 6.06 (Community 2). The non-tolerant ecotype showed effects of increasing stress on their reflectance from t1 through t5, after which they were dead and their reflectance remained constant (Table 6.03). The coverage of non-tolerant plants was reduced to zero by t13. The increase in coverage of tolerant plants was restricted so bare ground is left. This was then re-covered by the tolerant grass over time.

Figure 6.07 shows the effect a low level of metal contamination may have on community of 100% non-tolerant plants (Community 3). Plants experienced stress (model inputs changing as in Table 6.04) although their coverage was not reduced. At t6 a tolerant species of plant invaded, and outcompeted the non-tolerant plants for space. The coverage then changes until tolerant plants were at full cover, and non-tolerant plants were at zero cover. At no point was bare soil exposed.

Figure 6.08 shows the response of a community consisting solely of non-tolerant plants to high levels of metal input (Community 4). Their cover was reduced, and with no tolerant plants present only bare soil remained (t11 - t12). At t12 tolerant species of plant invaded, and increased its cover until it was at 100%.

With all parameters and proportions defined the model was run as shown in Figure 6.09. The reflectance of tolerant and non-tolerant plants were modelled separately according to the level of metal and time of measurement being modelled. Simple mixture modelling was used to calculate community reflectance from modelled plant canopy and soil reflectance (Fig. 6.09). The different components (non-tolerant, tolerant and soil) were considered independent and were added after being weighted by their respective cover fractions. This assumes a field of view recorded by the sensor as shown in Figure 6.10.

6.2.3 Analysis techniques

Reflectance results from a sample of sequences through time were compared using community reflectance, red edge position (REP) and vegetation indices. REP was measured using the first derivative of reflectance, which was smoothed with a width of 21 (Savitzky and Golay, 1964). The indices chosen were one developed in this study (652/605 - Hardy), Blackburn's PSSRa 800/675 and PSSRb 800/650 indices and Carter's 694/760 (Carter) index. They were chosen as they were all successful to some extent at differentiating a stress response in the leaf and canopy level experiments (Chapters 4 and 5).

6.3 Results

6.3.1 Monocultures

The canopy reflectance model outputs with no community mixing were analysed using REP and VI's. As there was no mixing these can be considered to be monoculture responses. The reflectance response for these inputs used to model non-tolerant plants responses to high stress shows reflectance greatly increasing across the spectrum with time (Figure 6.02). At t6 the plants are dead and soil becomes the dominant influence on reflectance. The non-tolerant plants modelled response to low stress show a slight increase in reflectance across the spectrum (Figure 6.03). Increases are larger away from the absorption maxima of chlorophyll and water. There was a slight increase in reflectance in the high metal treatment in tolerant plants relative to the control with high stress (Figure 6.04). Low stress reflectances were the same as control values.

Non-tolerant plants in response to both high and low stresses had REP moving to shorter wavelengths (Figure 6.11). The control REP was at 721 nm. The high treatment showed a greater movement (to 696 nm) than the low treatment (to 709 nm). The tolerant ecotypes REP moved from 721 nm in control and low treatments, to 716 nm in high treatments. The vegetation indices response for non-tolerant plants to low stress showed that the two PSSR indices had a stronger relationship with stress than the other indices (Figure 6.12). However, the PSSR indices moved to higher values with initial stress. In response to high stress all the indices from the literature responded clearly to stress (Figure 6.13). The index developed in this study showed a poorer relationship with stress than the others.

6.3.2 Community 1

Results

The change in modelled canopy reflectance with stress and community composition for a community of 99% non-tolerant plants and 1% tolerant to low levels of stress (Fig. 6.05) is given in Figure 6.14. This shows the change in reflectance with time at evenly spaced intervals. With the initial stress reflectance of the non-tolerant plants increased, especially away from the major absorbance features. From t8 onwards there was no further change in the stress responses of the plants, but community composition did change. The increase in proportion of tolerant plants reduced reflectance until it was the same at t20 as it was at t0. This was because model input parameters for the control non-tolerant plants were equal to the parameters for the tolerant plants at low levels of metal.

The modelled community composition and the results of red edge position (REP) and indices are given in Figure 6.15. The REP showed a fairly steady decline from 721 nm to 708 nm with the initial stress event until t4 (Fig 6.15B). REP stayed at 708 nm until t10 despite a further change in model inputs from t4 to t5, and changes in community composition from t4 onwards. After t10 tolerant plant cover increased past 50%. REP jumped from 708 nm at t10 to 716 nm at t11, then stayed there until t15 when it moved to 720 nm. The VI's seemed to track changes better, all are shown as a proportion of their values at t0 (t0 = 100; Fig 6.15C). The Hardy index showed only a slight change with stress and community composition changes. The other indices show a good response with stress moving away from the control value until t5. After the effect of dilution of the stress spectral signal by an increasing proportion of tolerant plants is evident until full tolerant cover at t20 returned the indices back to their original values.

Discussion

With no bare soil patches in the community view reflectance in this community was a result of stress changes in non-tolerant plants up to t5, after which the stress was at the same level. The community composition changed with an increase in the presence of tolerant plants from t5. All techniques (REP and VI's) showed an initial response to stress. The REP was slow to return to longer wavelengths with increased tolerant cover. It also had a stepped return indicating that small changes in community reflectance that pass certain thresholds can results in large changes in REP. The PSSR and Carter indices showed the best overall response, although both PSSR indices did not have a consistent change in values with stress as they increased in value initially and then decreased with additional stress. The Hardy index showed a slight response as indicated by the monoculture results (Fig 6.12). The change in proportion of ecotypes can be considered to be a dilution of the stress signal from non-tolerant plants by the unstressed tolerant plants. This is in agreement with the VI results, all of which show a gradual change back to their original values.

6.3.3 Community 2

Results

The reflectance response of a community (Fig. 6.06) comprised of 99% non-tolerant and 1% tolerant to high levels of stress showed a great deal of change with time (Figure 6.16). By t4 reflectance at all wavelengths had increased, although the spectrum was still recognisable as a vegetation response. At t8 non-tolerant vegetation was dead and decreasing in cover, tolerant vegetation (12%) was slowly covering the area leaving a significant amount of bare soil (38%). The reflectance spectra at t8 was not too different from that of bare soil, and had changed only a little by t12. By t16 tolerant vegetation cover had increased to 54%, and some spectral features of vegetation appeared in the spectrum. At t20 the community was 100% tolerant plants, and the reflectance was similar to that of non-tolerant plants at t0 (control).

The community composition changing through time with REP and VI results is shown in Figure 6.17. REP showed a decrease in wavelength position from 721 nm to 696 nm with stress from t0 to t5 (Fig. 6.17B "REP" series). After t5 the non-tolerant plants were dead and had a similar reflectance as bare soil (Fig. 6.02 t6 data), so the only major effect on community reflectance was the increase in tolerant plants cover. With the increase in tolerant cover from t5 to t11 (from 6% to 21%) REP moved from 696 nm to 718 nm. From t11 to t18 tolerant cover continued to increase, although REP stayed at 718 nm; after t18 it decreased to 716 nm.

All vegetation indices except Hardy showed a stress response between t0 and t5 (Fig. 6.17C). The scale of this response was much higher than in the low metal treatment. After t5 the main effect on community reflectance was the increase in proportional cover of tolerant plants. At low cover (<50%) both PSSR indices showed no response to increasing cover. The Carter index showed a slight response to increasing cover. With proportional cover of tolerant plants >50% the PSSR and Carter indices showed a stronger shift in index values back to near to their original positions.

Discussion

This community had the added complication of dead plants (from t6 and on) and bare soil (from t1 to t19) being part of the composite reflectance signal. REP showed a strong response to stress, and quickly increased to longer wavelengths with increasing tolerant plant cover compared to Community 1's lag. However it did not reach the wavelength position of 100% tolerant coverage until t19, indicating that soil coverage was affecting REP. The influence of soil reflectance on REP was tested by measuring REP for a simple community comprised of tolerant plants and soil only. Plant cover was decreased from 100 % to 0%, with soil replacing it (data not shown). Raw soil reflectance was used as well as the same soils data smoothed using a 41 point moving average. REP with smoothed soil reflectance did not move from the position it was at with 100% cover until plant cover was less then 12.5%, after which the red edge peak was indistinguishable. This is what would be expected with REP being invariant with plant cover (Horler *et al.*, 1983). Using the raw soil data, however, REP moved from 716 nm to 701 nm with decreasing plant cover. The variability in soil reflectance with wavelength is obviously confusing the red edge position in the modelled communities, although it had no effect on the output of the PROSAIL model (data not shown).

This effect was also investigated using Community 2's data. The raw soil data was smoothed with a 41 point moving average and REP was calculated (Figure 6.17B "Smooth" series). During the initial period of stress this showed a similar pattern to the results using raw soil data (<t5). Community REP with smoothed soil moved to longer wavelengths with increasing tolerant plant cover in a less jerky manner. With the smoothed soil REP also reaches the position it is at for 100% tolerant cover at t13, when cover by non-tolerant dead vegetation reached 0. REP with natural soil

spectra (Fig. 6.17B "REP") was therefore initially determined by stress (<t5). Between t5 and t13 it was determined by an increase in cover of soil and healthy tolerant plants, and decrease in cover of dead non-tolerant plants. After t13 REP was a result of soil reflectance and increasing cover by tolerant plants, of which the latter became the only modifying factor on REP only after t19 when they are at 87% coverage. This soil induced modification of REP was a result of the soil reflectance fluctuating across its spectrum, thus having its own REP response which modifies the vegetation's REP when mixed with it.

VI's from the literature showed a good initial response to stress, while the index developed in this study showed no response (Fig. 6.17C). All three literature indices then tracked the growth of the tolerant canopy on the site, rather than solely indicating its stress/chlorophyll level. If these indices were just responding to plant stress/chlorophyll content these indices should have held at one value once tolerant plants were the only plants in the community (>t13). VI's did not show any change in value from those displayed when smoothed soil reflectance data was used (data not shown).

6.3.4 Community 3

Results

The reflectance of this low stress 100% non-tolerant community increased with the initial stress event (to t5) especially away from absorption maxima (e.g. in the green and NIR; Figure 6.18). After t5 the only effect on reflectance came from the invasion by tolerant plants at t6 and their dominance of the community by t20. Reflectance decreased until t20 when it was identical to that at t0. The REP showed a rapid though step-like response to initial stress (Figure 6.19B). After t5 the only change was in community composition, and the REP stayed at short wavelengths until t14 when tolerant plant cover exceeded 50%. REP then rapidly increased in a step like manner to its final position, 721 nm. The VI's showed an initial response to stress, although the PSSR indices changed direction of movement with increased stress. After t5 all indices moved towards their original values with changing plant cover. There is a threshold at t14 (tolerant cover > 50%) where all indices accelerated their return to their original values.

Discussion

There was no bare soil to confuse the spectral response of this community. The initial response was best shown by the REP and Carter's index. The other indices either changed little (Hardy) or did not move in a consistent direction with stress (PSSR indices). The only factor in deciding the community reflectance after t5 was community composition. The change in proportion of ecotypes diluted the stress spectral response of non-tolerant plants as the tolerant plants were not stressed. The REP showed a poor relationship with this indicating that a few thresholds in reflectance values influenced its position, the main one being when >50% of plants in the community were tolerant. Until the thresholds were reached there was no movement, and once they were passed REP movement was large. The VI results showed a better response with dilution of the stress signal. As the proportion of tolerant plants increased stress responses were diluted further so VI's showed a gradual return to their control values. This accelerated once tolerant plants were dominant.

6.3.5 Community 4

Results

The reflectance response of a community comprised of only non-tolerant plants which was exposed to high levels of stress and then invaded by tolerant plants (Fig. 6.08) is given in Figure 6.20. Reflectance at all wavelengths increased with stress at t4. By t8 the plants were dead and their cover was reduced, so reflectance was very similar to soil reflectance. At t12 the plots were bare of vegetation. Tolerant plants invaded and the reflectance spectra showed slight pigment and water absorption features at t16. At t20 the community was 100% tolerant plants with a reflectance spectra very similar to non-tolerant plants control reflectance.

The REP and VI results changing through time with community composition are shown in Figure 6.21. REP moved to shorter wavelengths immediately with stress (Fig. 6.21B "REP" series). By t5 it had moved from 721 nm to 695 nm, after which all the plants were dead and so REP was unobtainable. Tolerant plants invaded at t13, and REP was next identifiable at t15, with a 15% coverage of tolerant plants. REP increased to 717 nm, and decreased to 716 nm at t20, which is the REP of a full canopy of tolerant stressed plants.

The Hardy index once again showed little response to stress. The other indices responded to the initial stress (<t5), and then showed no response because all the plants in the community were dead (Fig. 21C). Following the invasion of tolerant plants Carter's index responded first, then the PSSR indices. All three show a gradual change in index values despite the stress/chlorophyll level of the plants in the canopy not changing.

Discussion

This community was fairly simple, non-tolerant plants became stressed, died and their cover was reduced. Tolerant plants then invaded, and their cover increased. REP and the three indices from the literature showed a strong response to the initial stress. Then because the community only consisted of dead plants all indices showed no change . The lag following the invasion by tolerant plants (t13) before REP settles (t16, cover at 32%) and the discrepancy between this REP and the REP at 100% cover (as the plant characteristics aren't changing the REP should remain the same) again reveals the influence of soil on REP. This was tested using the same technique as Section 6.3.3; soil data was smoothed with a 41 point moving average (Fig. 6.21B "Smooth" series). This allowed the interpretation of REP at t14, sooner following the invasion by tolerant plants, and REP also stayed constant until full cover. However, smoothing also affected the REP response to the initial stress event, delaying its move to shorter wavelengths slightly. VI's from the literature responded to stress, but responded to the change in cover with the invasion by tolerant plants rather than the stress/chlorophyll content of those plants.

6.4 Conclusion

This chapter investigated the responses of different communities where a contamination event occurs. A plant stress response in ecotypes of different tolerance was modelled, as well as possible community responses to plant stress. These were combined to give the reflectance of different communities in response to stress. VI's and REP were calculated to assess the use of those techniques for detecting contamination in these communities. All VI's from the literature and REP showed a good initial response to stress. With an increase in cover of tolerant unstressed plants the stress signal from the non-tolerant plants decreased in a close relationship with proportional cover for the VI's. The REP showed a step like response to an increasing proportion of unstressed plants in the community, indicating that it was invariant until it reached thresholds. VI's and REP response to the increase in cover in the stressed communities of tolerant unstressed plants was confused by any changing fractional cover of bare soil. With an increased proportional cover of bare soil VI's showed a change in their values equivalent and to that of a stress signal . REP was influenced by the soil spectra fluctuating with wavelength, which affected the REP when the plant and soil spectra were mixed. If the background soil reflectance was more linear (smoother), these effects did not happen and REP would be a good indicator of stress regardless of percentage soil cover.

All VI's from the literature used a visible and a near infra-red waveband. With stress the difference in reflectance between these bands decreases. With decreasing plant cover the same thing happens (Fig. 6.16 as an extreme example). With a high plant cover these indices performed well. They tracked stress events and a decreasing proportion of stressed plants due to their replacement by tolerant plants (Figs. 6.15; 6.19). However, the response of indices for an unstressed canopy with decreasing plant cover was very similar to the stress response of a full canopy (Figure 6.22). Without decoupling the response to stress from the response to cover these indices would be of little use in the field (Steven *et al.*, 1992). The index developed in this study (GP/RT) showed the same response to increasing soil cover, though it did not respond to stress as well as the other indices.

The REP was affected by decreasing plant cover because of soil reflectance. When the soil reflectance increased smoothly with wavelength REP was a useful technique, and responded as reported by (Horler *et al.*, 1983) with no change over different covers. It did not respond smoothly with canopy stress however, which was particularly noticeable when the proportion of unstressed plants increased slowly (e.g. Fig. 6.15B). However the actual soil reflectance spectra fluctuated with wavelength, and when mixed with the plant reflectance affected the REP. The existence of this effect in natural systems would vary from soil to soil, and may have been an artefact of the mixing procedure. The presence of thresholds in REP response could come from the relative importance of short and long wavelength shoulders (Chapter 3, Section 3.4.2; (Horler *et al.*, 1983)). A small change in canopy physiology could make one shoulder more important, so shifting the REP a lot. However, the modelled first derivative results did not show two shoulders.

Modelling is a useful tool for investigating relationships, and suggesting questions to ask but not necessarily answering them. The success is dependent on the accuracy of the model and the inputs fed into it. The models used here have been validated against real data (Goel, 1988; Jacquemoud *et al.*, 1996). The inputs were based on biological understanding of tolerance and stress, and were within ranges used by other authors (Table 6.01; 6.02). The models showed that vegetation indices used here and REP would be appropriate for the detection of contaminated land in some situations. The assumption would have to be made that soil cover is not changing if VI's are used, or that soil has a near liner reflectance with wavelength if REP is to be used. Very recent contamination events would have the highest chance that there is still a full cover of non-tolerant plants. If users require frequent stress assessments VI's or REP could be used. However, these responses will need study in the natural environment.

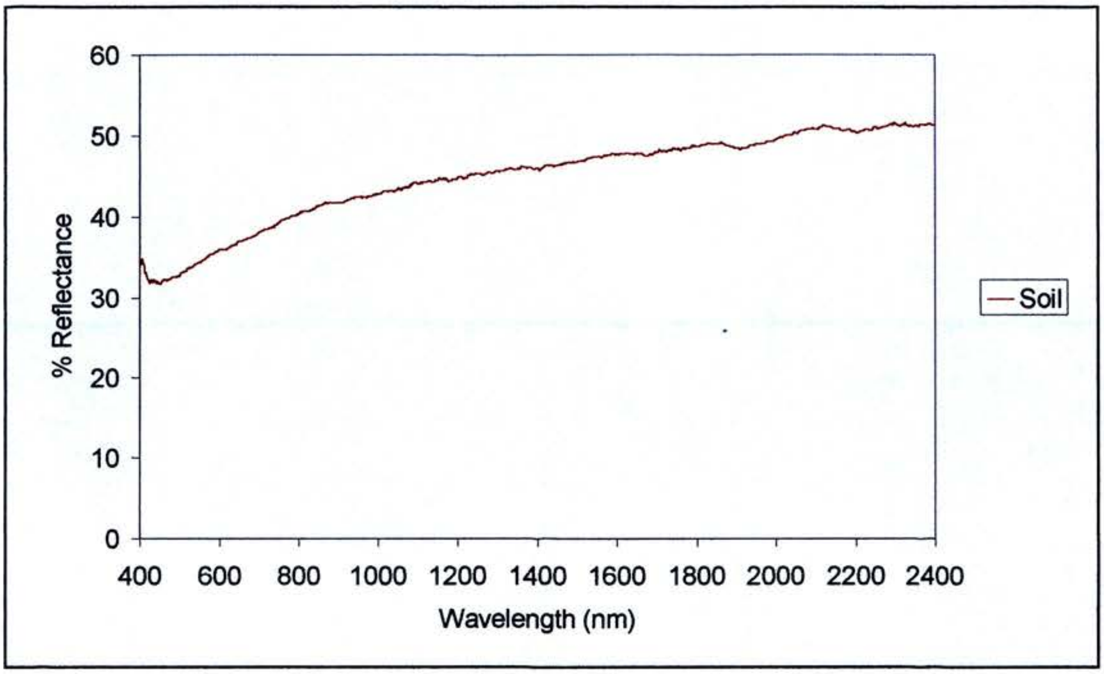

Figure 6.01. Soil reflectance data used for canopy and community modelling.

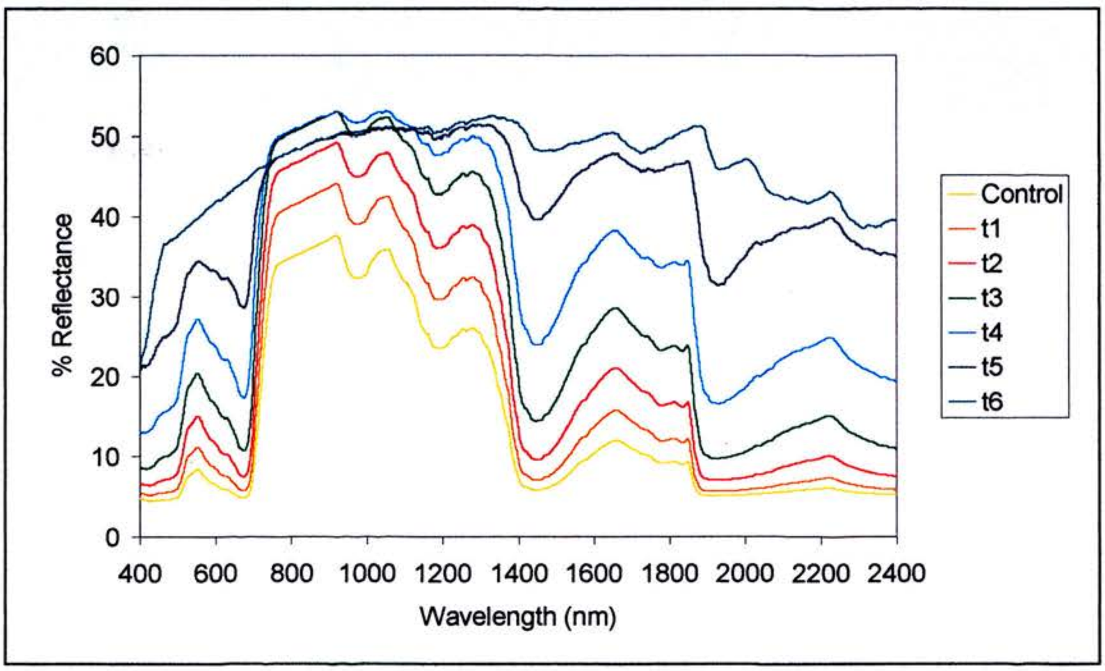

Figure 6.02. Modelled canopy reflectance for non-tolerant plants in response to high stress (Table 6.03)

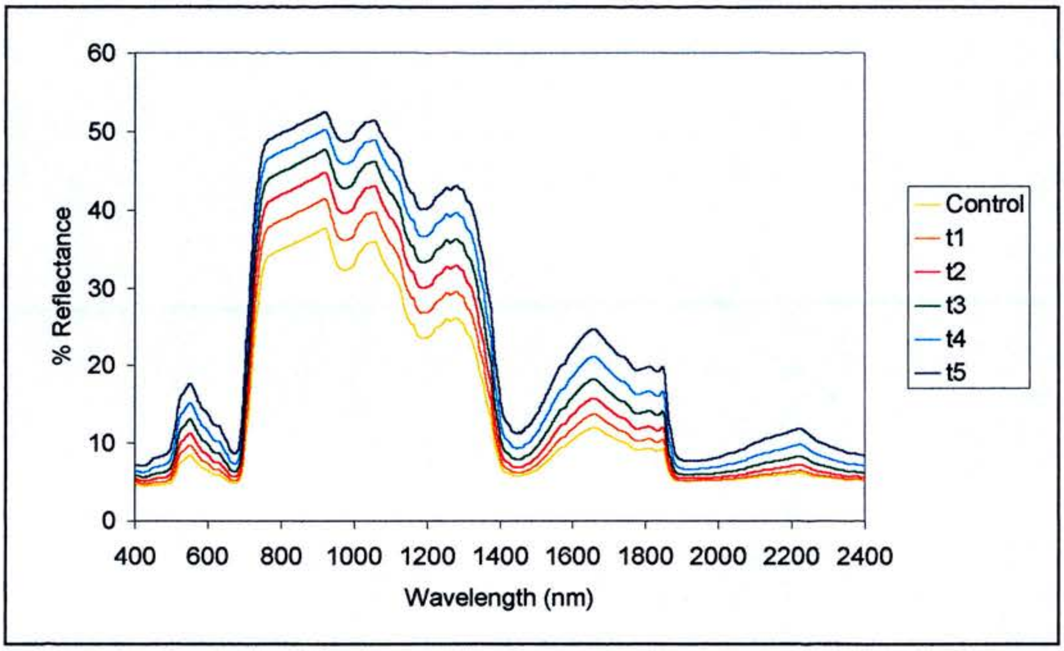

Figure 6.03. Modelled canopy reflectance for non-tolerant plants in response to low stress (Table 6.04)

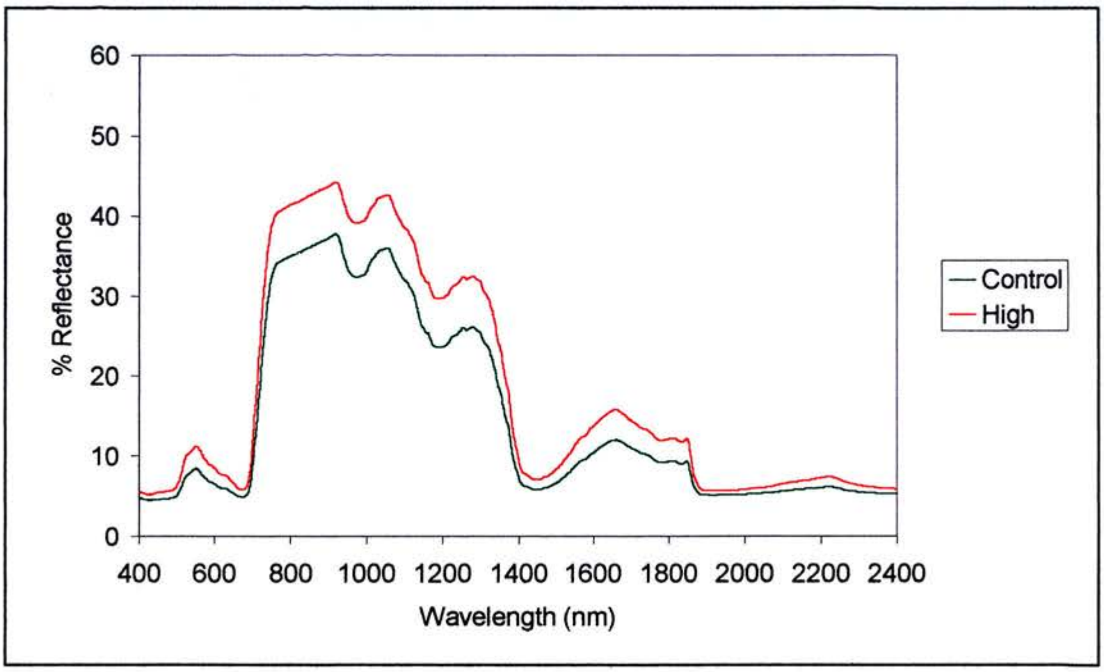

Figure 6.04. Modelled canopy reflectance for tolerant plants under control, low and high stress. Low stress results are the same as control values.

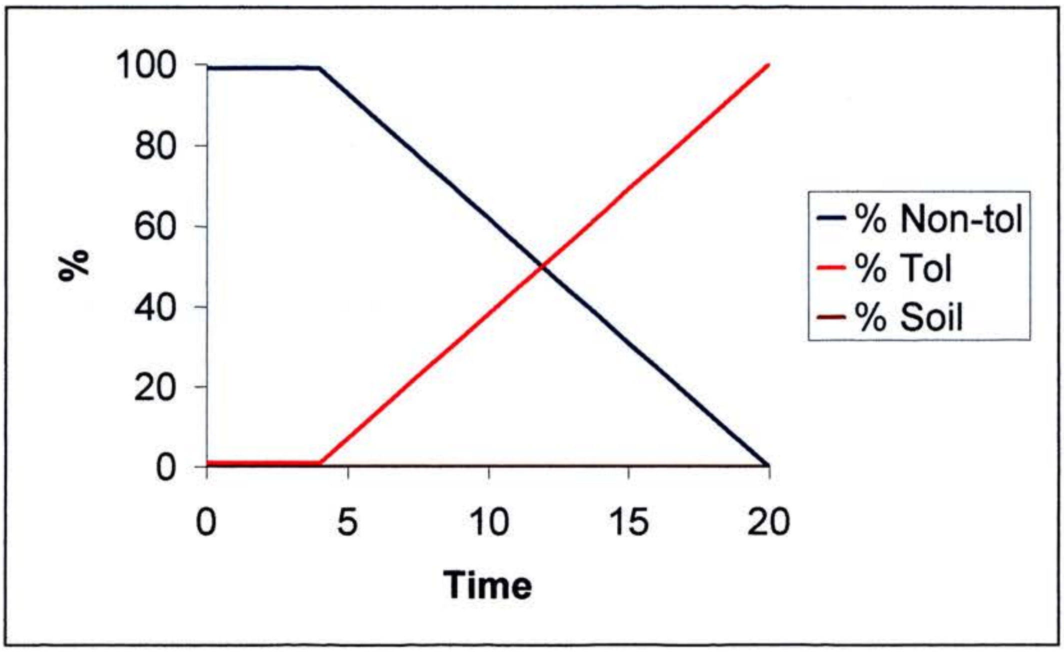

Figure 6.05. Change in Community 1's composition with time.

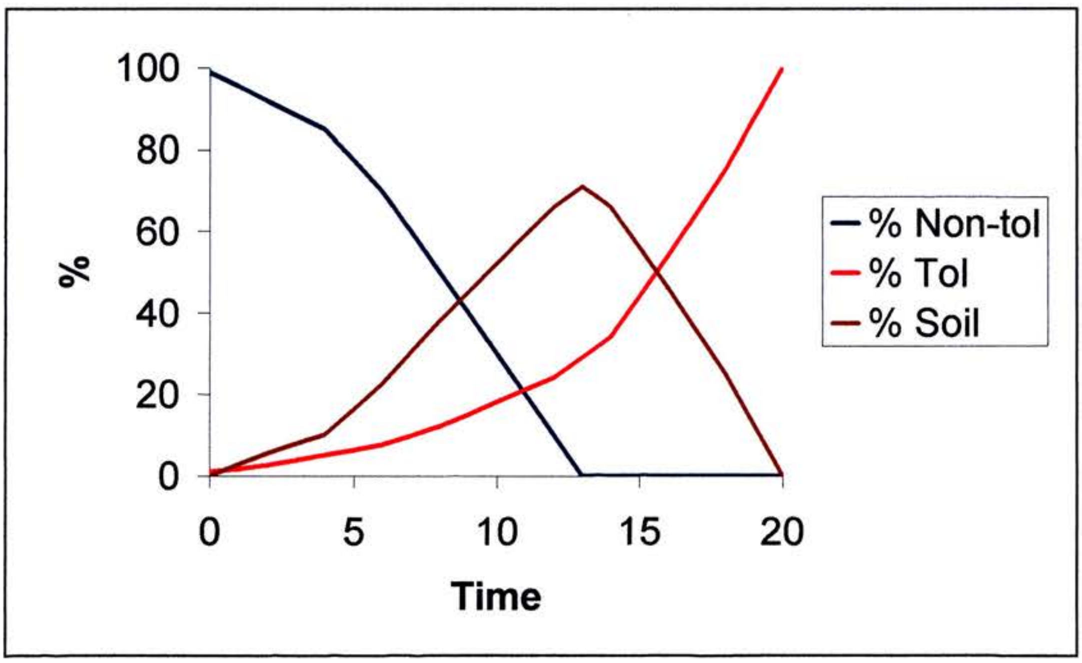

Figure 6.06. Change in Community 2's composition with time.

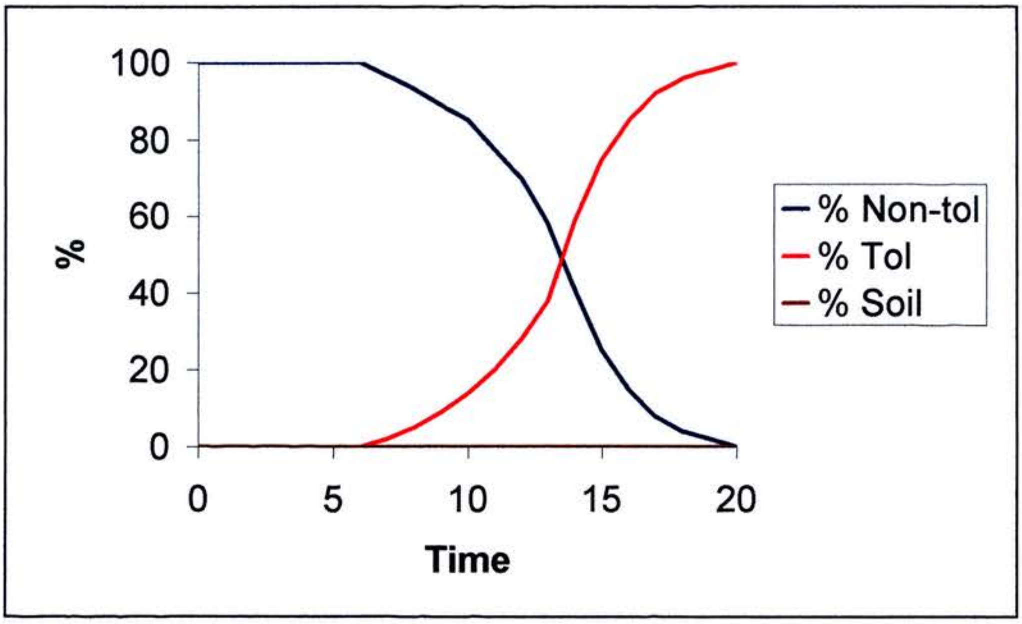

Figure 6.07. Change in Community 3's composition with time.

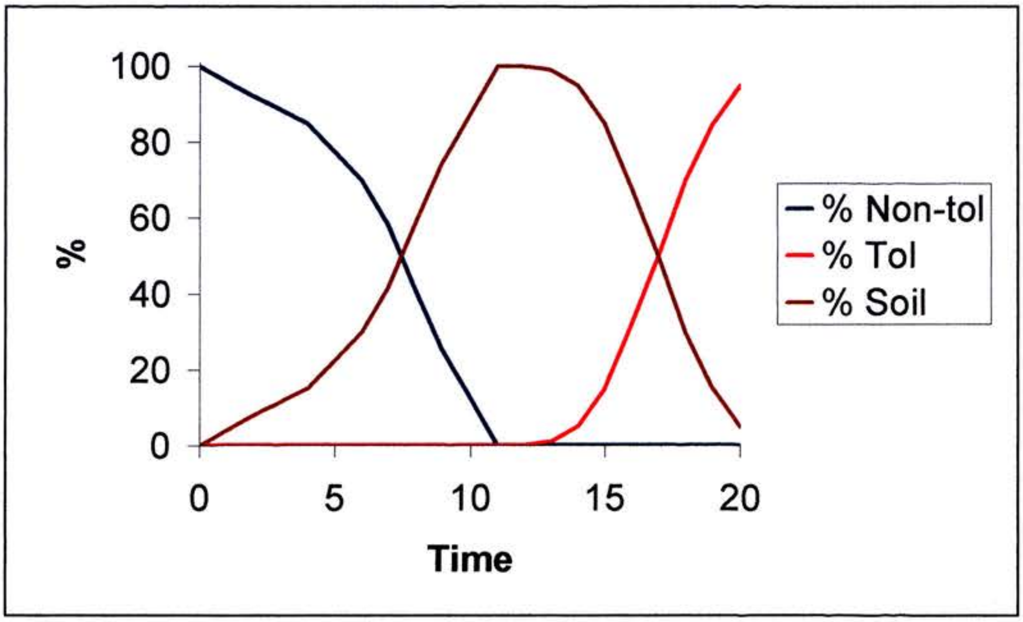

Figure 6.08. Change in Community 4's composition with time.

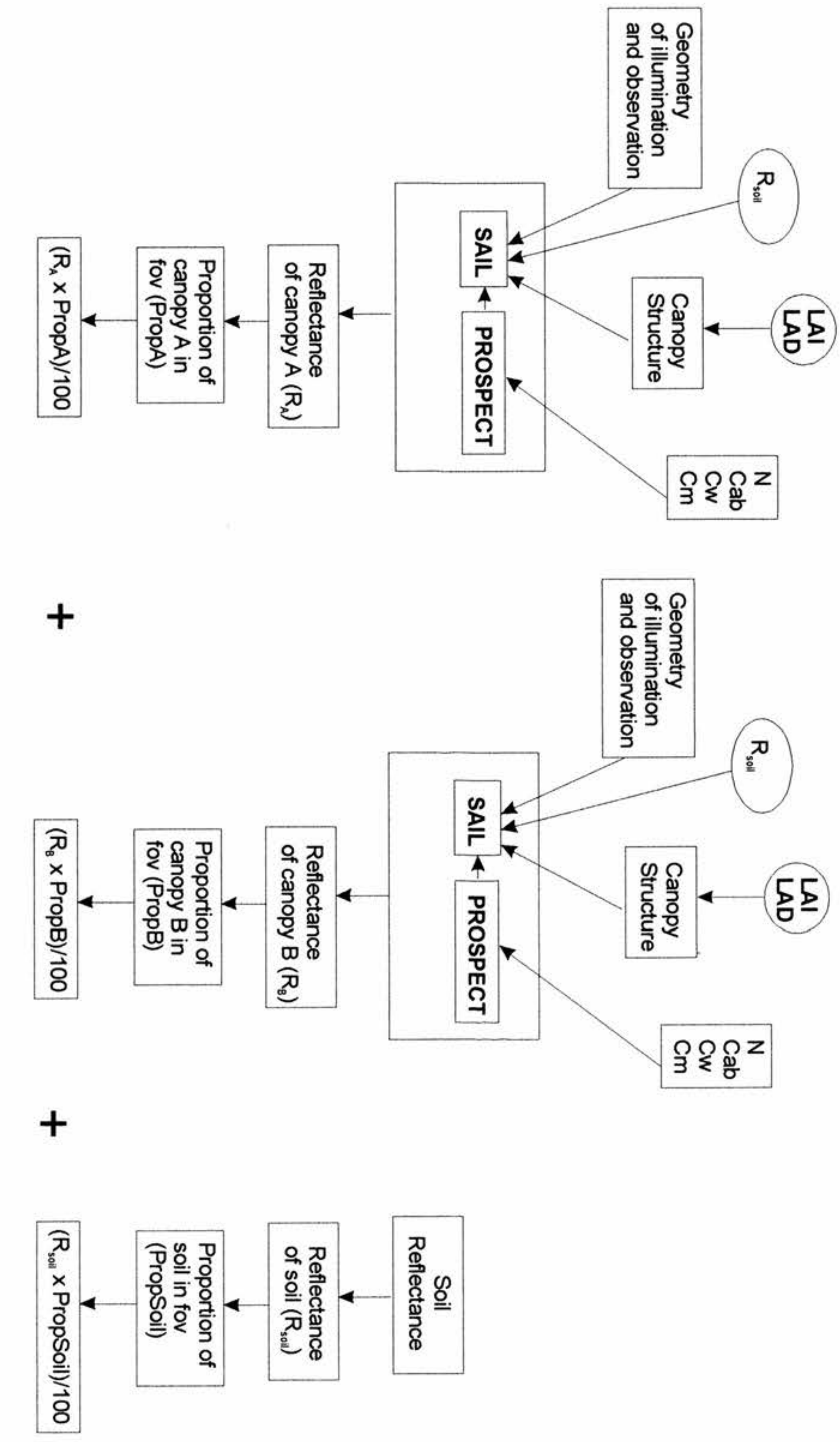

Figure 6.09. Flow diagram showing how the community model was run based on the combined PROSAIL model and community composition

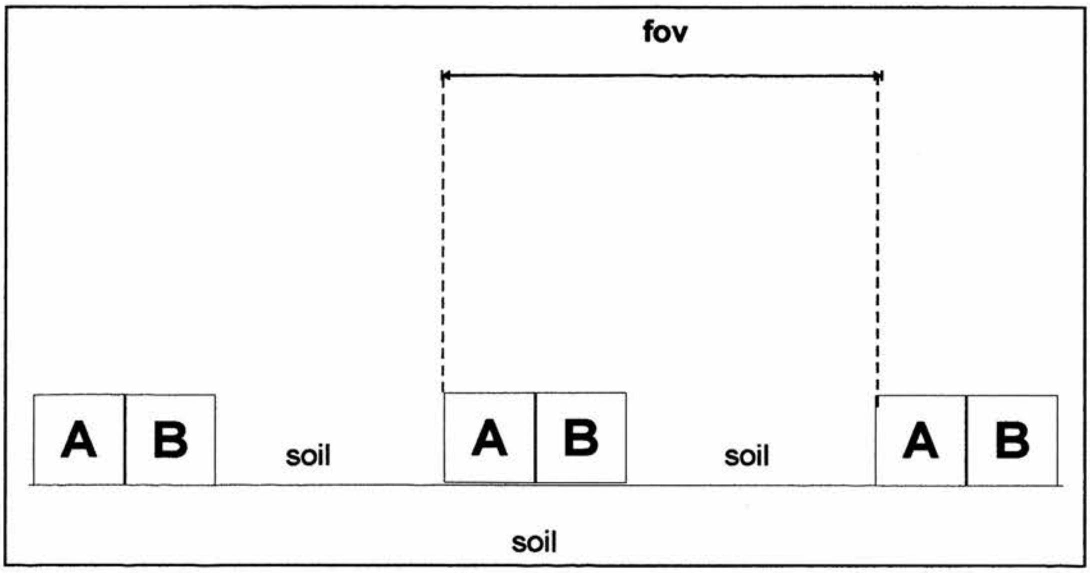

Figure 6.10. The additive component modelling created this field of view (fov) for the sensor. The fov is a composite of the proportion of canopies of two ecotypes (A and B) and soil.

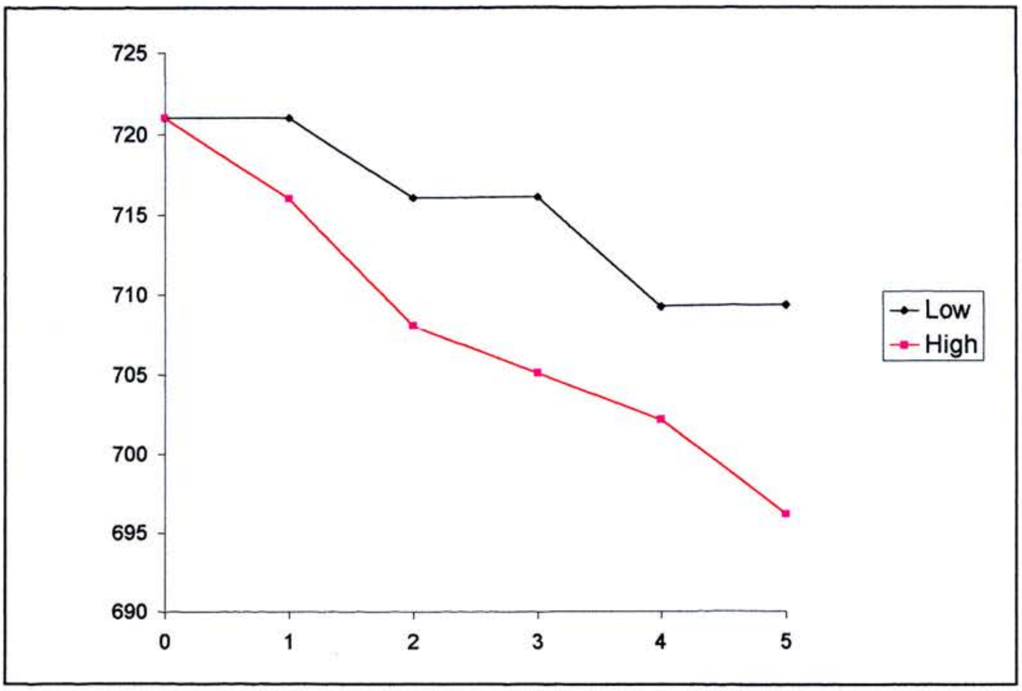

Figure 6.11. Change in REP for non-tolerant monocultures in response to stress.

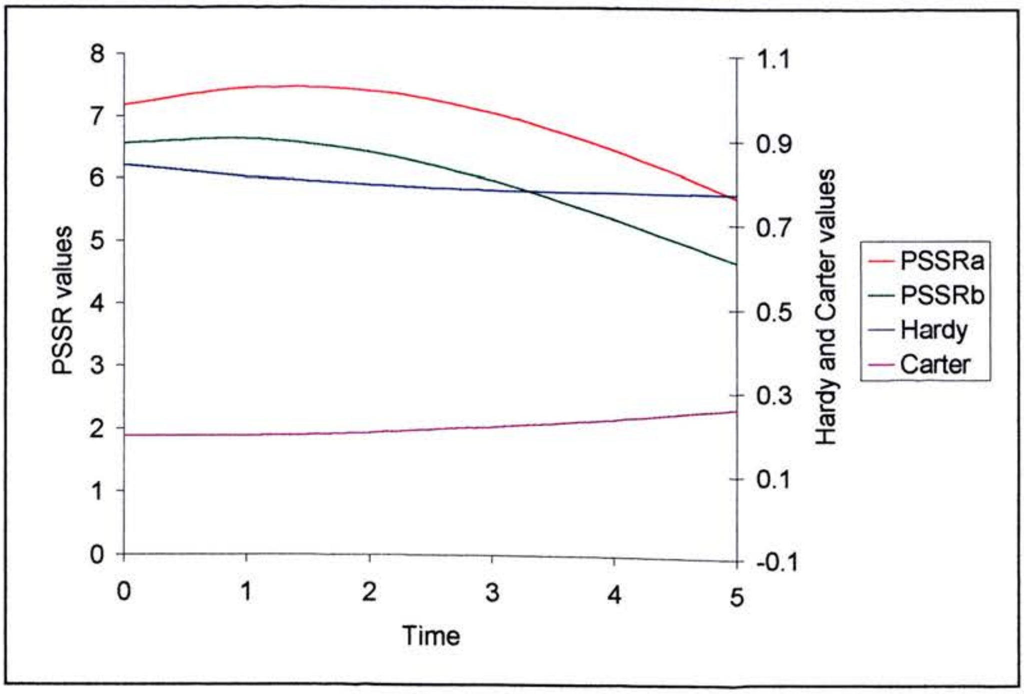

Figure 6.12. Vegetation index results for non-tolerant monocultures in response to low stress.

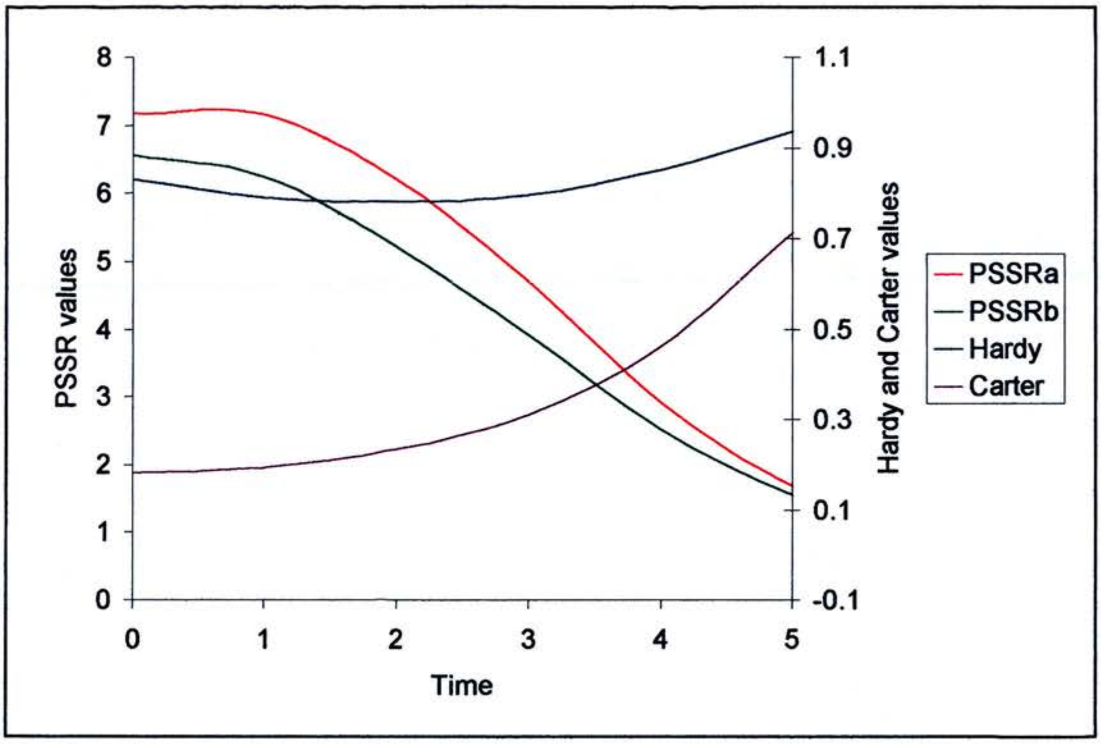

Figure 6.13. Vegetation index results for non-tolerant monocultures in response to high stress (Table 6.03).

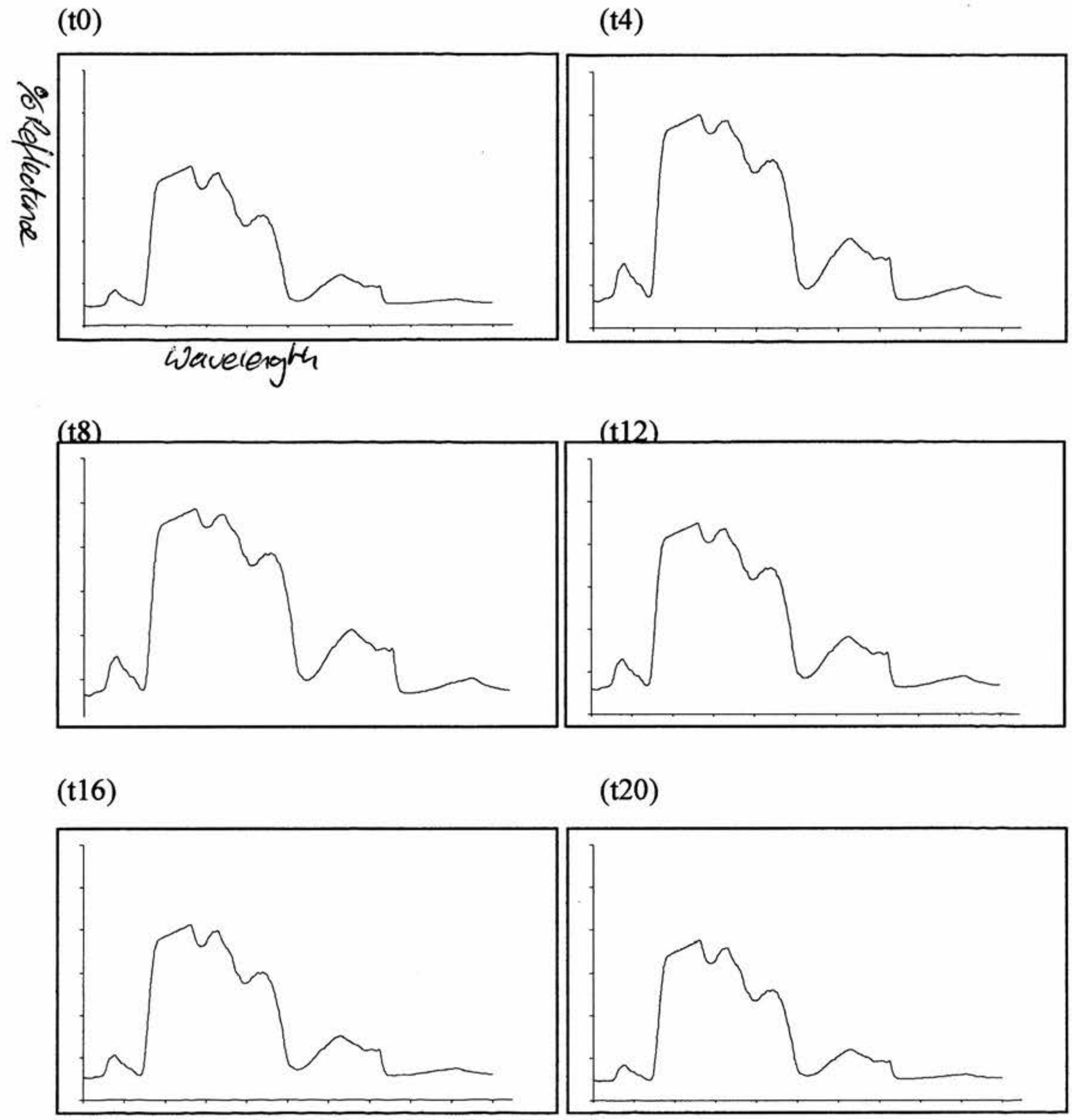

Figure 6.14. Reflectance response of Community 1 with time.

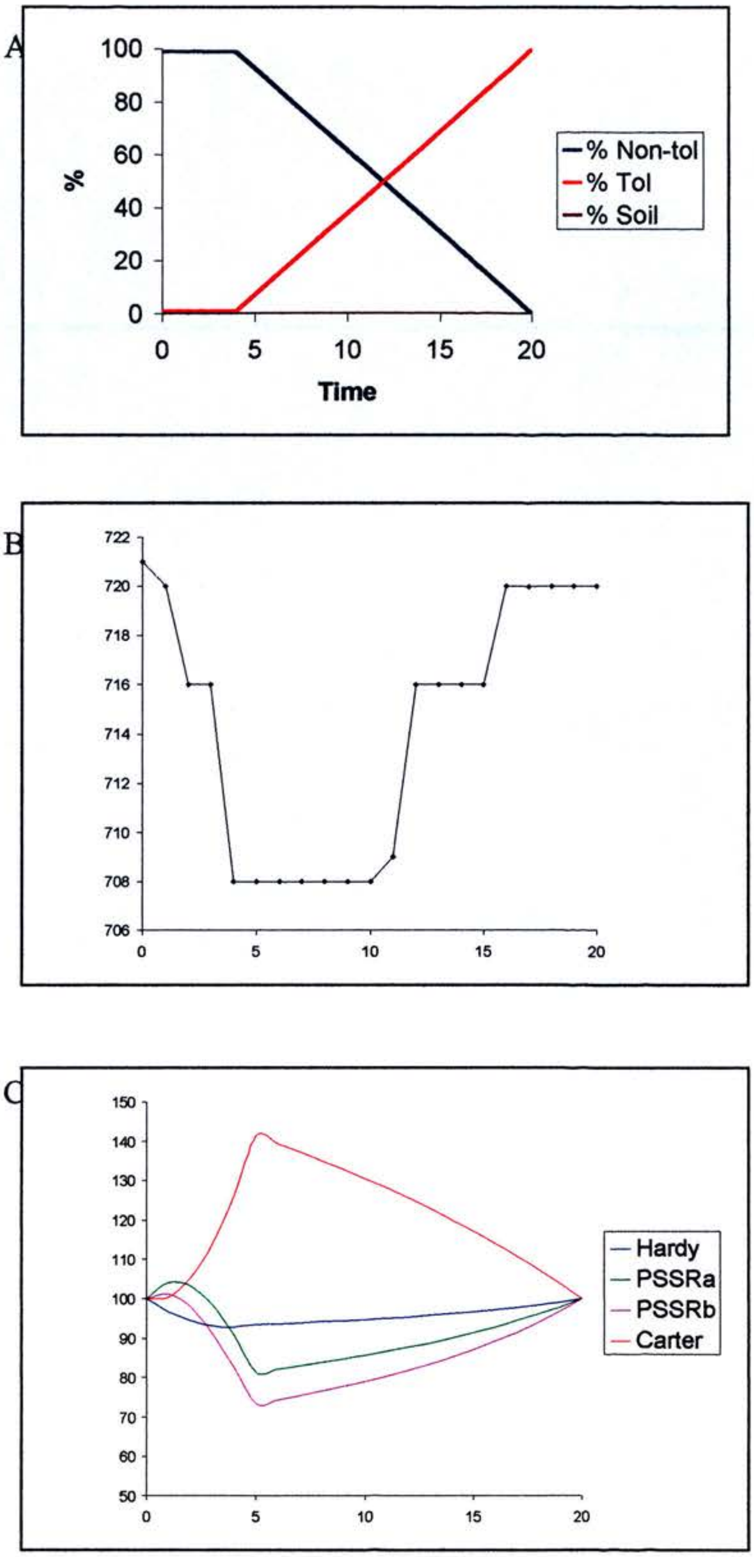

Figure 6.15. Community composition (A), REP (B) and VI responses (C) for Community 1 with time. In C, VI responses are shown as a proportion of their values at t0.

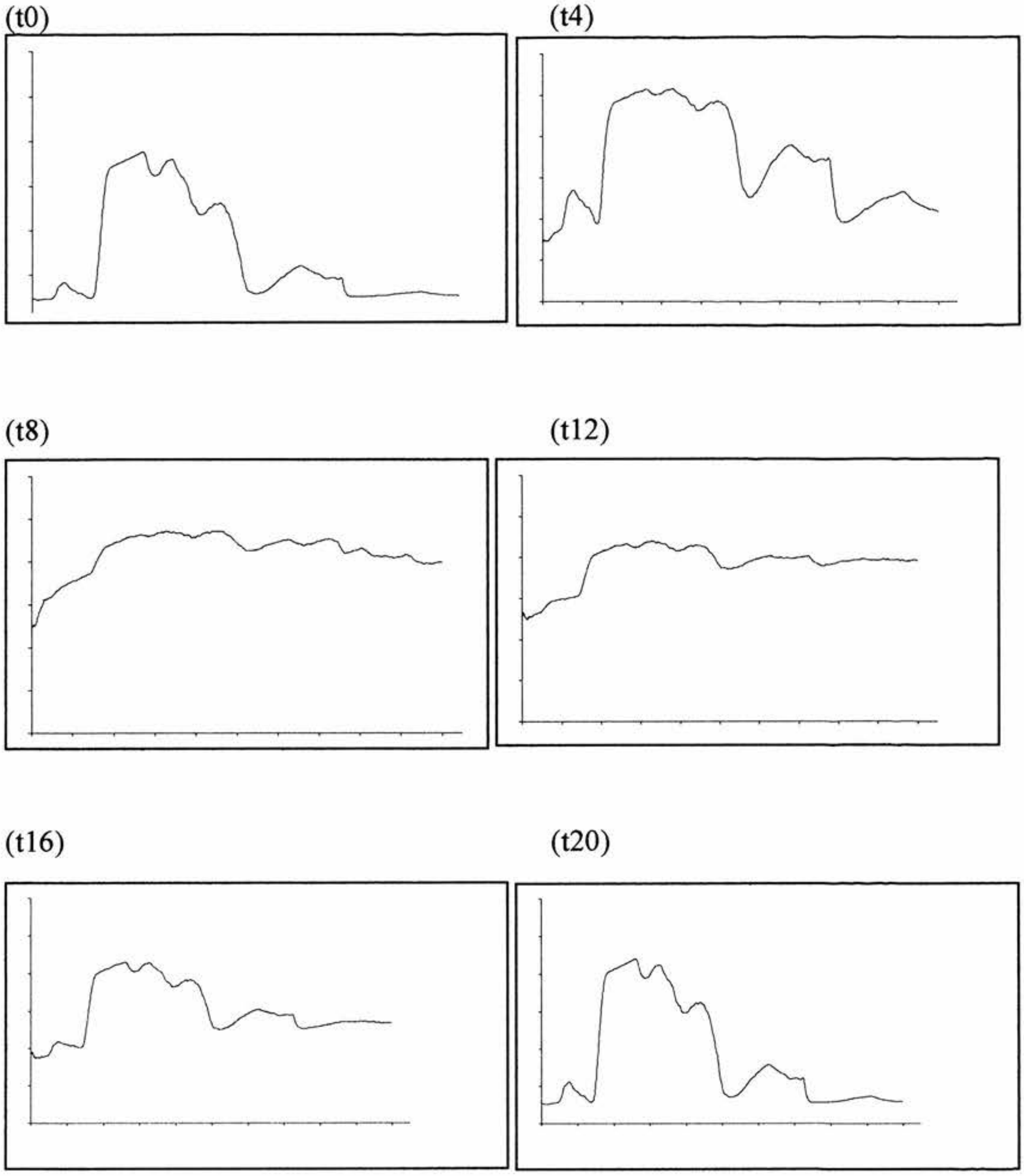

Figure 6.16. Reflectance response of Community 2 with time.

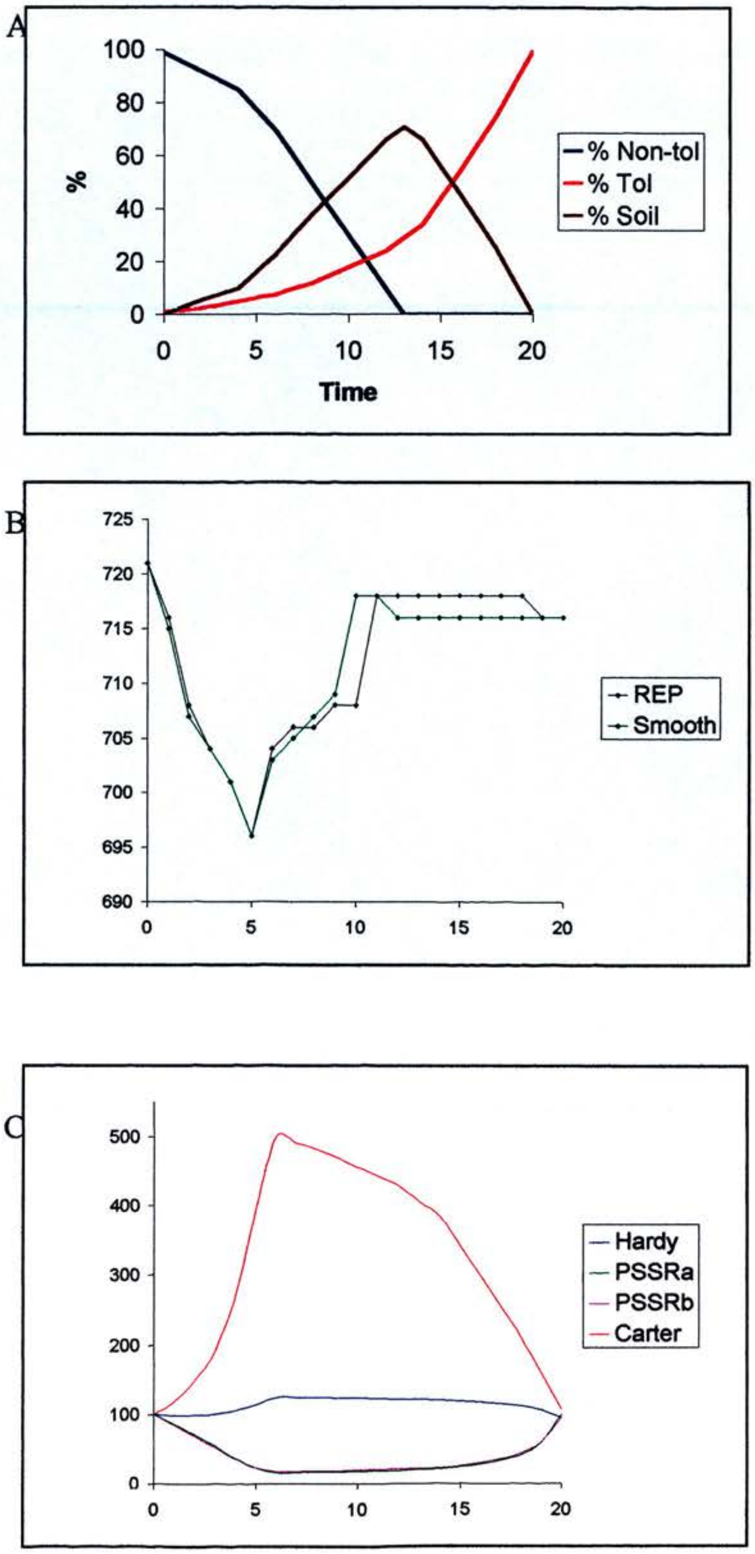

Figure 6.17. Community composition (A), REP (B) and VI responses (C) for Community 2 with time. In C, VI responses are shown as a proportion of their values at t0.

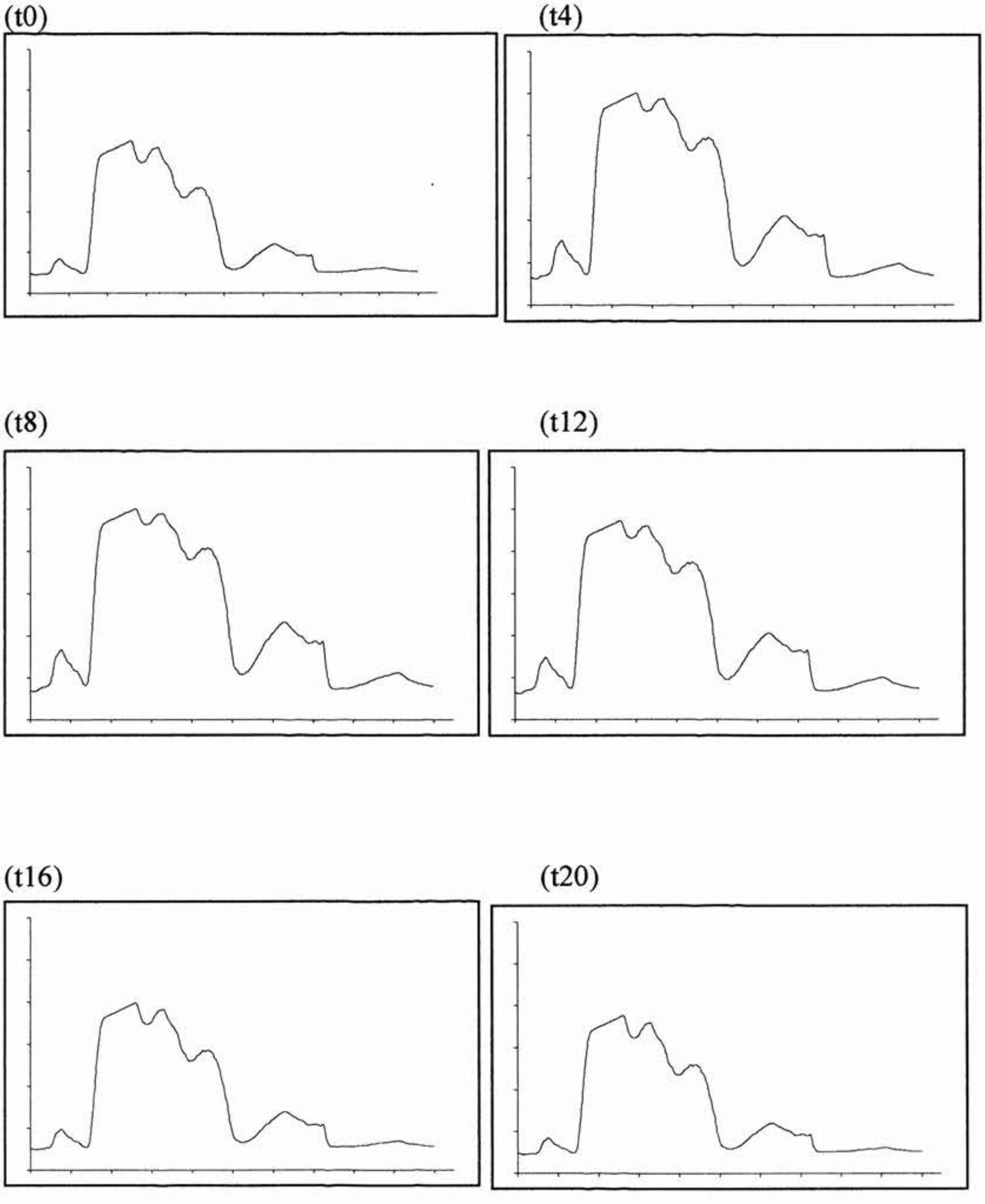

Figure 6.18. Reflectance response of Community 3 with time.

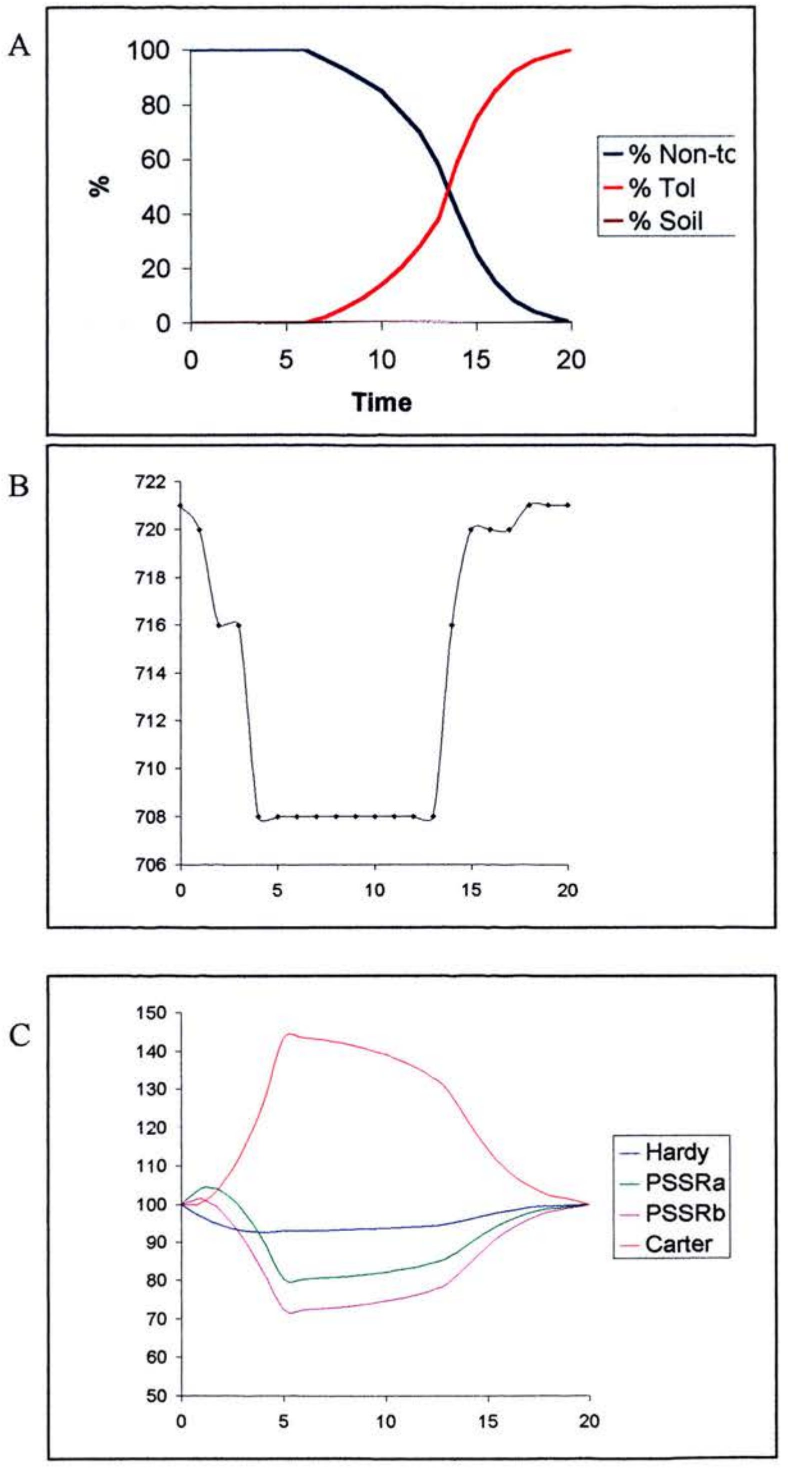

Figure 6.19. Community composition (A), REP (B) and VI responses (C) for Community 3 with time. In C, VI responses are shown as a proportion of their values at t0.

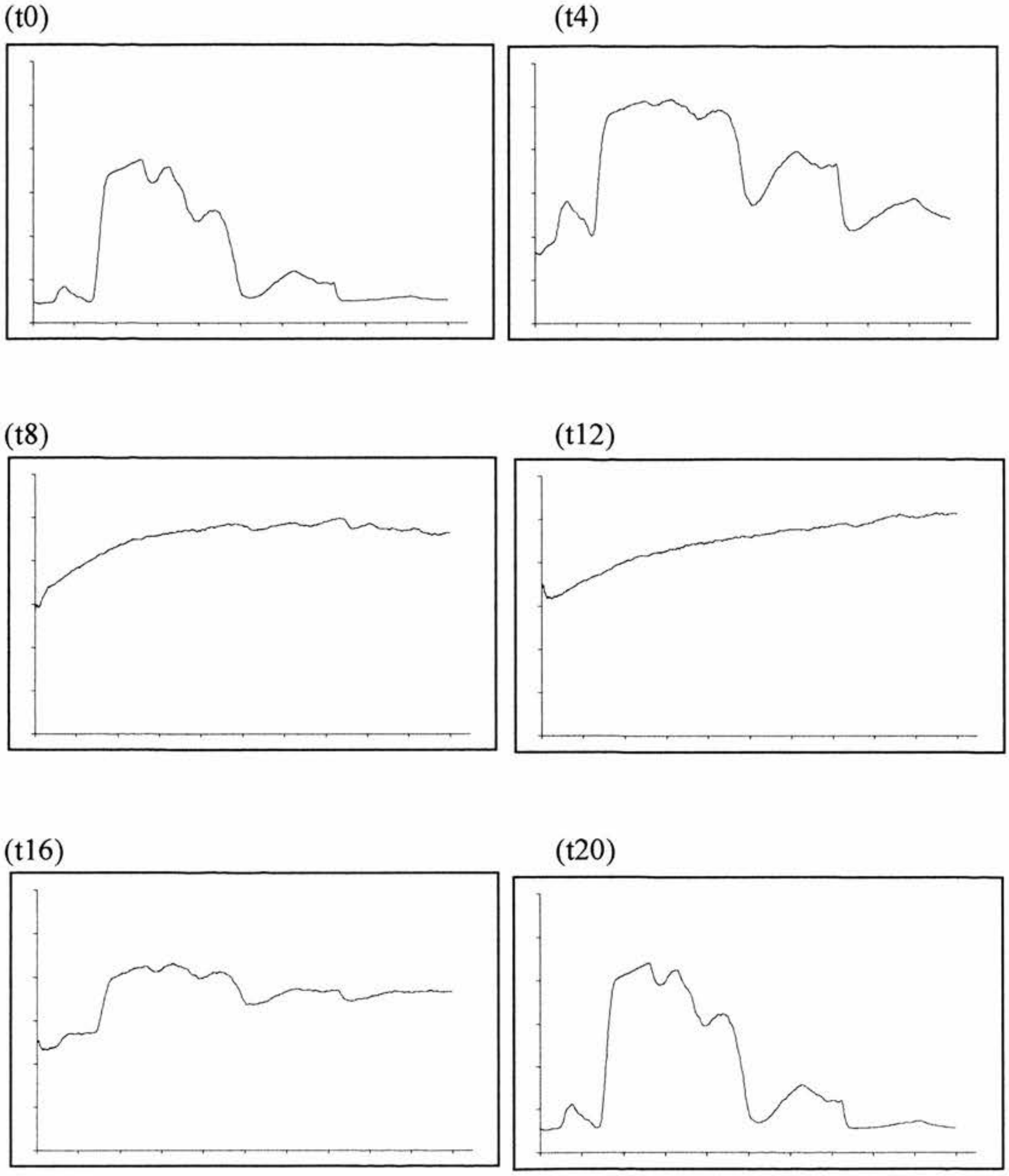

Figure 6.20. Reflectance response of Community 4 with time.

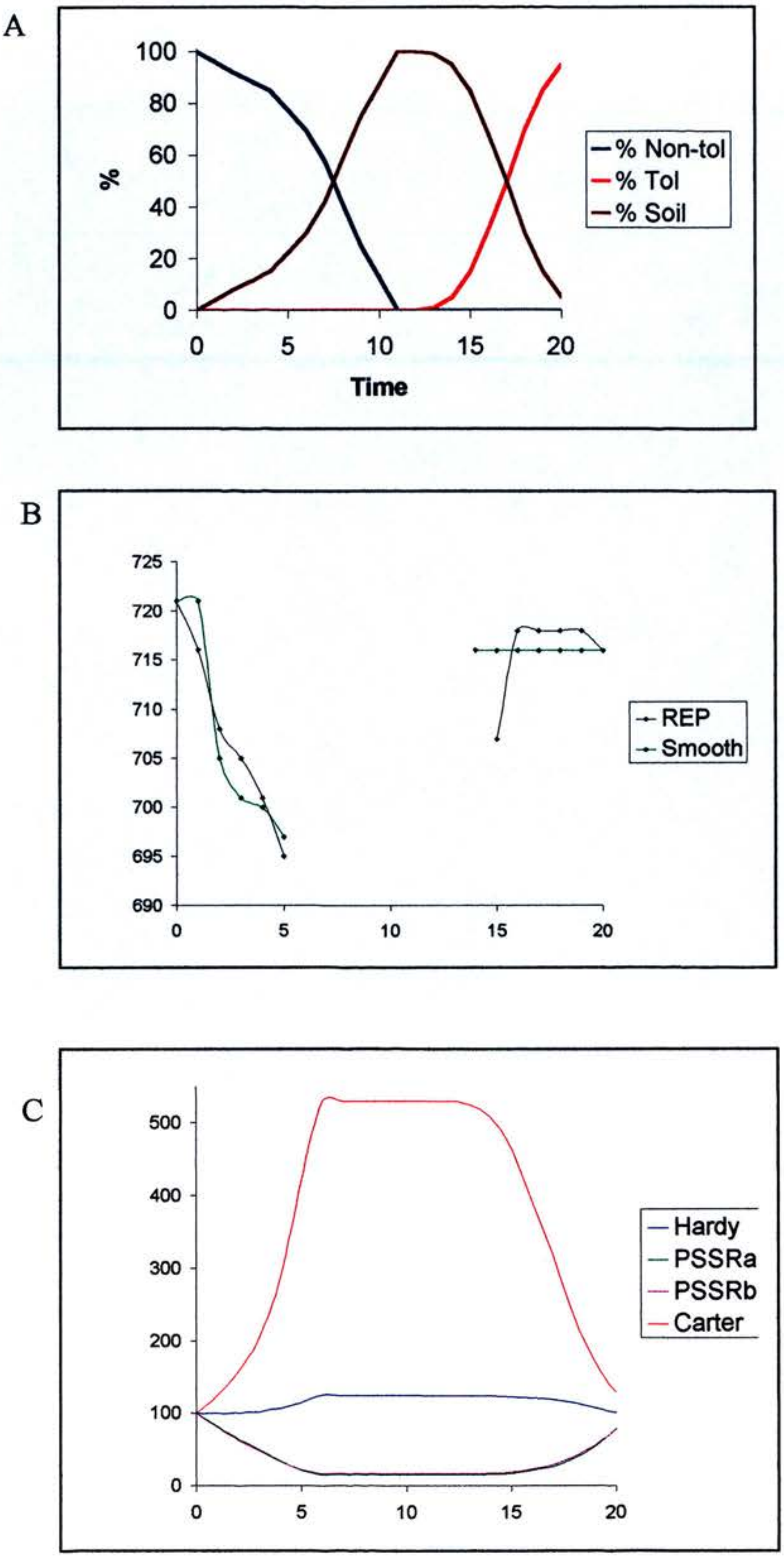

Figure 6.21. Community composition (A), REP (B) and VI responses (C) for Community 4 with time. In C, VI responses are shown as a proportion of their values at t0.

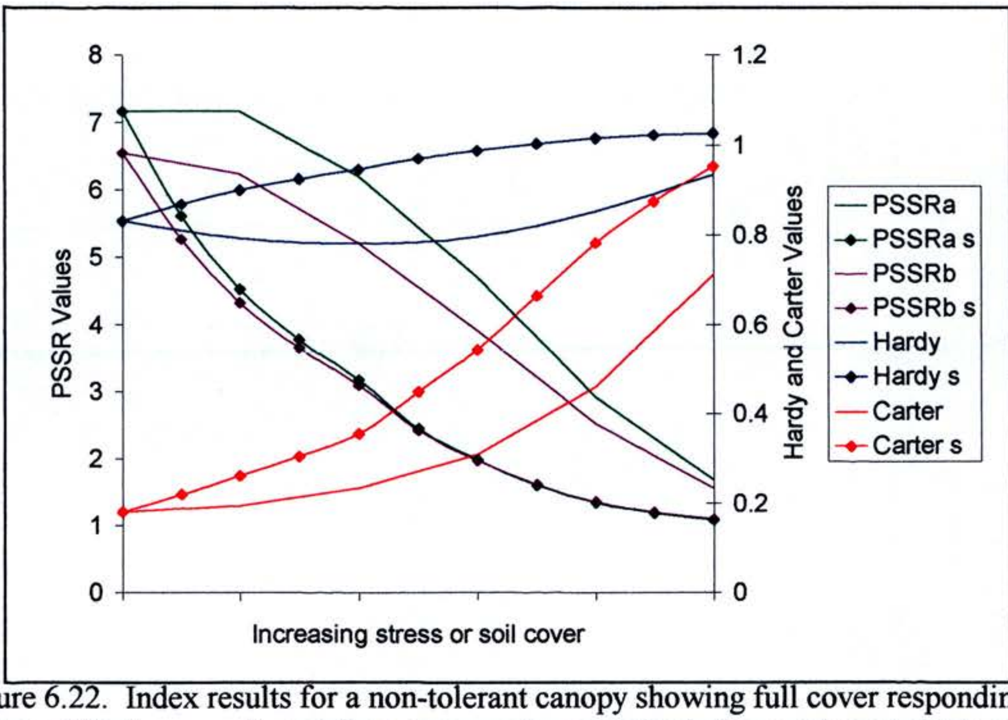

Figure 6.22. Index results for a non-tolerant canopy showing full cover responding to stress ("*Index name")*, and for a community comprised of non-tolerant plants and soil with soil cover increasing ("*Index name* s"). With a changing soil cover the indices responded in a similar manner to stress.

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Chapter 7: Conclusions

This study aimed to investigate the influence of plant tolerance on leaf and canopy spectral responses to contamination. The detection of contaminated ground by remote sensing would be useful for many applications, but it's reliance on biomarkers of contamination needed testing. This is the first study that has considered the influence of tolerance on the ability of remote sensing to detect contaminated ground via vegetation stress.

Two ecotypes, one tolerant to high metal concentrations and one non-tolerant, of *Festuca rubra* and *Agrostis capillaris* were grown in controlled conditions with different concentrations of zinc, copper and salt. Leaf reflectance was measured using an integrating sphere, and chlorophyll *a* and *b* concentrations were taken. Reflectance, vegetation indices (VI's) and red edge position (REP) were used to investigate whether there was a stress response. For all ecotypes, reflectance and VI's showed a difference between the control and all treatments. However, each VI distinguished different treatments in different ecotypes, and none could be applied to reliably distinguish all differences where they occurred in all ecotypes. REP only distinguished some treatments from the control. Different ecotypes of the same species had different responses to the same stress, not necessarily related to their tolerance.

Tolerant and non-tolerant ecotypes of *Festuca rubra* were also grown in larger areas with different levels of zinc contamination to measure the canopy reflectance response to stress. The ecotypes were grown in monoculture and mixtures. A moveable, artificially lit tent was used to measure canopy reflectance, and concomitant chlorophyll *a* and *b* measures were taken. Reflectance, VI's and REP were assessed for suitability in detecting contamination. There was much less response of VI's and REP from monoculture plots than for the mixture plots. Different ecotypes had different reflectance responses to stress which were not necessarily related to their tolerance. The timing of measurement was important in determining the results. The most obvious stress response was during senescence where non-tolerant stressed plants senesced before non-stressed plants.

A PROSPECT-SAIL model was combined with simple community models to investigate the ability of remote sensing to detect contamination in the natural environment. This suggested that the only time remote sensing could be used to locate contamination was during the initial period of stress, as the community would be non-tolerant, and at full cover. VI's were affected by stress and changing vegetation cover. REP was affected by stress and bare soil cover.

Remote sensing, in the form of high spectral resolution reflectance measurements performed in a spectrophotometer, was successful at distinguishing stressed leaves from non-stressed leaves. However, leaf responses did not scale up to canopy level responses. Monoculture canopies showed little reflectance response to metal inputs. The presence of stress varied with the contaminant, the plant species and ecotype. The stress, therefore, did not relate to the metal concentration in the soil. Photosynthetic pigment concentration had little influence on leaf reflectance, and did not vary with applied stress in different ecotypes. This experiment suggests that even a direct measure of pigment concentration would not distinguish metal contaminated sites on certain occasions.

Given the few spectral responses to stress that were discovered, the suitability of the dosage applied to the plots has to be considered. There have been many leaf stress studies (including this one - Chapter 4) that have discovered many leaf reflectance responses to stress. However, this project's canopy study uncovered few (Chapter 5). One of the aims of this study was to simulate the natural environment as closely as possible while maximising control over experimental variables. Canopy reflectance studies on metal stress in the natural environment have concluded that stress-induced differences in timing of senescence are the best chance in detecting contamination (Bell and Labovitz, 1985; Saraf *et al.*, 1989). Given that this research project arrived at similar conclusions the metal level applied seems valid. This was confirmed as mixture canopies showed a more obvious reflectance response to stress despite less metal being applied to them, perhaps because they were measured slightly later in the season. Fertiliser applications may have decreased the effect of metal doses (Nagy and Procotor, 1997). The slow growth on the plots meant that measurements had to be made late in the season, measurements throughout the season would have been preferable. This experiment would be improved by having more replicates.

As inferred above, the experimental design used throughout this study enabled control over the treatments. Field sites would have been influenced by plant and soil heterogeneity. This much simplified system allowed the only difference between plots to be the ecotype and metal concentration. The variation between members of the same ecotype would be minimal given the controlled sources of the seed. In the natural environment each plant would likely have a different response to metal. Each plant would also be exposed to different soil characteristics. This experimental approach made the testing of the theoretical basis of remote sensing of vegetation stress easier, but divorced the study from the natural environment. By being controlled the experimental plots offered the best case scenario for remote sensing of metal contamination. That this study still failed to find a method that could successfully locate contaminated ground via stress would indicate that in the natural environment where the soil metal content, plant tolerance and percentage ground cover vary within a scene the use of remote sensing as an exploratory tool would be limited. Different ecotypes showed different responses to different stresses. Increasing bare soil cover caused VI's to respond as if there was increased stress. While the relationship between REP and bare soil is a minor one, it could affect possible thresholds in plant REP responses.

For remote sensing to reliably and routinely identify contamination via its effect on plant canopies a universally present causal link is required between soil metal concentration, plant stress, and plant spectral response (Chapter 1, Figure 1.01). Soil chemistry affects the bioavailability of metals. Plant stress is a function of the bioavailability of metals and the physiological characteristics of the plant, which are inherently variable. Some plants are more tolerant than others, so a similar stress response between plants will not be equivalent to the same metal content. Plant canopy spectral response varies with leaf, canopy and soil optical properties, and the relationships between these and reflectance are not fully understood. It is at the level of the plant that the relationship between metal contamination and plant reflectance breaks down the most. Metal bioavailability is the most useful measure of contamination for most purposes, as total metal content reveals little about a metal's effects (Ross, 1994; Marschner, 1995), so a measure of metal bioavailability would be adequate. Plant spectral responses to different stresses are generally similar (Carter, 1993), so if it is accepted that the cause of the stress would not be identifiable this is still adequate. However, plants of different tolerances to stress will negate the required relationship between soil metal and plant spectral response.

Having a library of species and their spectral responses to stress has been proposed to identify contamination in the field (Clark *et al.*, 1995). This may be useful in agriculture where species identity would be known and unmixed. In the natural environment, however, where communities are mixed, species identity is unknown and ecotypes of the same species vary in response the extensive research necessary to document responses to stress would be of no value. Even if remote sensing could distinguish species, or if they were known on a particular site, ecotypes could not be distinguished.

Figure 1.01 introduced the ways in which the use of remote sensing to detect contaminated ground may break down. This study showed that within the same species populations exist that have different physiologies. The same spectral response in different ecotypes was caused by different treatments. Remote sensing can still locate contaminated ground via vegetation stress in certain cases. Such situations may include, for example, the recent contamination of a site, or contamination where tolerant plants have not invaded, or areas where tolerant plants are stressed. Remote sensing would detect these areas along with areas stressed by different factors (false positive results). Current understanding would suggest that detecting the cause will not be possible. Remote sensing would not detect contaminated areas covered by tolerant vegetation (false negative results). Any user of remote sensing would have to be willing to use it as a preliminary search tool only, to be backed up by ground surveys with the knowledge that many contaminated sites would be ignored.

For remote sensing to accurately locate ground contamination by heavy metals, plants should ideally be at full cover in the period of their growth season where stress affects reflectance the most. This is most likely during senescence, where stressed plants may senesce before non-stressed. The identity of the species and ecotype should be known, and its stress spectral responses should be understood. The plants must not be tolerant to the contaminant. Thus, the opportunities for using remote sensing with accuracy are therefore very limited. The agricultural context is perhaps the one area where all of these factors requirements could be met, although not without extensive further research. The benefits of such information in modern agricultural systems would be a fraction of the previously visualised potential of remote sensing.

Future research should consider the variable responses of different non-tolerant ecotypes of the same species. Research should focus on canopy level responses, as leaf responses do not predict canopy responses. Stress detection methodologies that respond only to stress and not community composition should be developed.

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Appendix A: Statistical test results for vegetation indices, chapters 4 and 5

This section contains the results of statistical tests represented graphically in the relevant tables in chapters 4 and 5 (e.g. A4.14 here refers to Table 4.14).

Table A4.14. Results of Mann-Whitney U tests comparing vegetation index results from treatments individually to the control for *Fr* Jupiter (NT). * indicates that there is a significant difference.

			Comparison of treatment to control				
Author	Title	Index	Lo Cu	Hi Cu	Lo Zn	Hi Zn	Salt
Blackburn 1998b	PSSRa	800/675	16*	16*	11	16*	9
	PSSRb	800/650	16*	16*	16*	16*	16*
	ref. PSSRa	800/680	16*	16*	11	16*	9
	ref. PSSRb	800/635	16*	16*	8	16*	12
	PSSRc	800/500	16*	16*	8	16*	12
	PSNDa	(800-680)/(800+680)	16*	16*	16*	16*	16*
	PSNDb	(800-635)/(800+635)	16*	16*	16*	16*	12
Carter 1994		760/695	16*	16*	8	16*	15
Malthus et al 1995		425/470	16*	16*	16*	16*	11
		446/477	12	12	13	16*	16*
		541/836	16*	16*	12	16*	11
		818/538	16*	16*	12	16*	12
		818/713	16*	16*	16*	16*	9
Penuelas et al1994	WBI	970/900	10	16*	11	10	16*
	PRI	550-530/550+530	11	16*	10	10	10
	NPCI	680-430/680+430	16*	16*	10	16*	16*
Hardy	GP/RT	GP/RT	14	16*	16*	16*	16*

Table A4.15. Results of Mann-Whitney U tests comparing vegetation index results from treatments individually to the control for *Fr* Merlin (T). * indicates that there is a significant difference.

			Comparing Control to these treatments individually using MW-U test				
Author	Title	Index	Lo Cu	Hi Cu	Lo Zn	Hi Zn	Salt
Blackburn 1998	PSSRa	800/675	13	16*	14	16*	16*
	PSSRb	800/650	11	16*	12	16*	16*
	ref. PSSRa	800/680	12	16*	14	16*	16*
	ref. PSSRb	800/635	9	16*	14	16*	16*
	PSSRc	800/500	14	16*	14	16*	16*
	PSNDa	(800-680)/(800+680)	11	16*	11	16*	16*
	PSNDb	(800-635)/(800+635)	16*	16*	11	16*	16*
Carter 1994		760/695	9	16*	11	16*	16*
Malthus et 1995		425/470	11	15	11	16*	16*
		446/477	9	15	12	16*	16*
		541/836	8	14	8	16*	16*
		818/538	10	15	8	16*	16*
		818/713	10	16*	10	16*	16*
Penuelas et 1994	WBI	970/900	14	16*	12	16*	16*
	PRI	550-530/550+530	10	16*	8	16*	16*
	NPCI	680-430/680+430	12	16*	12	16*	16*
Hardy	GP/RT	GP/RT	10	16*	16*	16*	16*

Table A4.16. Results of Mann-Whitney U tests comparing vegetation index results from treatments individually to the control for *Ac* Coginan (T). * indicates that there is a significant difference.

			Comparing Control to these treatments individually using MW-U test				
Author	Title	Index	Lo Cu	Hi Cu	Lo Zn	Hi Zn	Salt
Blackburn 1998	PSSRa	800/675	16*	16*	16*	16*	16*
	PSSRb	800/650	16*	16*	16*	16*	16*
	ref. PSSRa	800/680	16*	16*	16*	16*	16*
	ref. PSSRb	800/635	16*	16*	16*	16*	16*
	PSSRc	800/500	16*	16*	16*	16*	16*
	PSNDa	(800-680)/ (800+680)	16*	16*	16*	16*	16*
	PSNDb	(800-635)/ (800+635)	16*	16*	16*	16*	16*
Carter 1994		760/695	16*	16*	16*	16*	16*
Malthus et 1995		425/470	16*	16*	16*	16*	16*
		446/477	16*	16*	16*	16*	16*
		541/836	16*	16*	16*	16*	16*
		818/538	16*	16*	16*	16*	16*
		818/713	16*	16*	16*	16*	16*
Penuelas et 1994	WBI	970/900	16*	16*	16*	16*	16*
	PRI	550-530/ 550+530	16*	16*	16*	16*	16*
	NPCI	680-430/ 680+430	16*	16*	16*	16*	16*
Hardy	GP/RT	GP/RT	16*	16*	16*	16*	16*

Table A4.17. Results of Mann-Whitney U tests comparing vegetation index results from treatments individually to the control for *Ac* Lance (NT). * indicates that there is a significant difference.

			Comparing Control to these treatments individually using MW-U test				
Author	Title	Index	Lo Cu	Hi Cu	Lo Zn	Hi Zn	Salt
Blackburn 1998	PSSRa	800/675	16*	16*	16*	16*	16*
	PSSRb	800/650	16*	16*	16*	16*	16*
	ref. PSSRa	800/680	16*	16*	16*	16*	16*
	ref. PSSRb	800/635	16*	16*	16*	16*	16*
	PSSRc	800/500	16*	16*	16*	16*	16*
	PSNDa	(800-680)/ (800+680)	16*	16*	16*	16*	16*
	PSNDb	(800-635)/ (800+635)	16*	16*	16*	16*	16*
Carter 1994		760/695	16*	16*	16*	16*	16*
Malthus et 1995		425/470	16*	16*	16*	16*	16*
		446/477	16*	16*	16*	16*	16*
		541/836	16*	16*	16*	16*	16*
		818/538	16*	16*	16*	16*	16*
		818/713	16*	16*	16*	16*	16*
Penuelas et 1994	WBI	970/900	16*	16*	16*	16*	16*
	PRI	550-530/ 550+530	16*	16*	16*	16*	16*
	NPCI	680-430/ 680+430	16*	16*	16*	16*	16*
Hardy	GP/RT	GP/RT	16*	16*	16*	16*	16*

Table A5.09. Vegetation Indices used to find a response to treatment, 5th Sep. Kruskal-Wallis statistical test results comparing control, low and high zinc treatments results for that index.

Author	Name of Index	Formulae	Jupiter (NT)	Merlin (T)
(Blackburn, 1998)	PSSRa	800/675	3.38	0.72
	PSSRb	800/650	3.38	0.56
	reformed PSSRa	800/680		1.34
	reformed PSSRb	800/635		1.58
	PSSRc	800/500	3.92	1.86
	PSNDa	$\frac{800-680}{800+680}$	3.38	0.72
	PSNDb	$\frac{800-635}{800+635}$	3.38	0.56
(Carter and Miller, 1994)		695/760	3.42	0.50
(Malthus *et al.*, 1995)		425/470	4.58	0.56
		446/477	4.82	1.26
		541/836	5.46	2.34
		818/538	5.46	1.34
		818/713	2.34	0.72
(Penuelas *et al.*, 1994)	WBI	970/900	5.18	2.06
	PRI	$\frac{550-530}{550+530}$	0.42	2.06
	NPCI	$\frac{680-430}{680+430}$	2.24	0.72
(Carter and Miller, 1994)		694/420	1.82	0.86
		600/760	3.42	1.58
		694/760	3.42	0.5
		VIS/760	3.38	1.22
(Dawson *et al.*, 1999)		NDVI	2.54	0.56
		SAVI	2.96	0.62
This Study	GP/RT	GP/RT	2.94	2.48
	GP/990	GP/990	5.46	1.26
	990/RT	990/RT	2.96	0.78
	652/605	652/605	1.68	2.88

*** indicates a statistically significant difference between treatments of the same ecotype with $p<0.05$.

Table A5.11. Vegetation Indices used to find a response to treatment, 16th Sep. Kruskal-Wallis statistical test results comparing control, low and high zinc treatments results for that index.

Author	Name of Index	Formulae	Jupiter (NT)	Merlin (T)
(Blackburn, 1998)	PSSRa	800/675	5.04	0.38
	PSSRb	800/650	3.84	0.96
	reformed PSSRa	800/680	4.02	0.24
	reformed PSSRb	800/635	3.02	1.46
	PSSRc	800/500	0.14	0.5
	PSNDa	$\frac{800-680}{800+680}$		
	PSNDb	$\frac{800-635}{800+635}$		
(Carter and Miller, 1994)		695/760	5.46	0.06
(Malthus *et al.*, 1995)		425/470	0	0.38
		446/477	0.74	0.42
		541/836	1.28	2.18
		818/538	1.46	1.82
		818/713	2.16	0.54
(Penuelas *et al.*, 1994)	WBI	970/900	5.04	0.02
	PRI	$\frac{550-530}{550+530}$	0.74	3.02
	NPCI	$\frac{680-430}{680+430}$	0.02	0.38
(Carter and Miller, 1994)		694/420	5.46	0.26
		600/760	1.52	2.06
		694/760	5.46	0.08
		VIS/760	1.46	0.62
(Dawson *et al.*, 1999)		NDVI	3.38	0.26
		SAVI	3.38	0.98
This Study	GP/RT	GP/RT	9.14*** a	1.14
	GP/990	GP/990	1.34	1.22
	990/RT	990/RT	4.46	0.56
	652/605	652/605	9.14*** a	0.08

*** indicates a statistically significant difference between treatments of the same ecotype with $p<0.05$.

"a" indicates that multiple comparison testing found a significant difference between the control and high zinc treatments.

Table A5.13. Vegetation Indices used to find a response to treatment 15th Oct. Kruskal-Wallis statistical test results comparing control, low and high zinc treatments results for that index.

Author	Name of Index	Formulae	Jupiter (NT)	Merlin (T)
(Blackburn, 1998)	PSSRa	800/675	4.94	2.94
	PSSRb	800/650	4.58	3.26
	reformed PSSRa	800/680	4.56	3.02
	reformed PSSRb	800/635	4.86	2.78
	PSSRc	800/500	0.06	1.82
	PSNDa	$\frac{800-680}{800+680}$	4.56	3.02
	PSNDb	$\frac{800-635}{800+635}$	4.86	2.78
(Carter and Miller, 1994)		695/760	5.04	2.48
(Malthus *et al.*, 1995)		425/470	1.62	2.22
		446/477	1.52	1.04
		541/836	1.34	1.26
		818/538	1.58	1.26
		818/713	5.66	2.06
(Penuelas *et al.*, 1994)	WBI	970/900	2.88	1.26
	PRI	$\frac{550-530}{550+530}$	2.22	2.48
	NPCI	$\frac{680-430}{680+430}$	5.46	1.68
(Carter and Miller, 1994)		694/420	5.66	1.28
		600/760	2.16	3.14
		694/760	5.04	2.34
		VIS/760	3.44	2.54
(Dawson *et al.*, 1999)		NDVI	3.5	3.02
		SAVI	3.5	3.02
This Study	GP/RT	GP/RT	9.68*** a	3.5
	GP/990	GP/990	2.66	0.98
	990/RT	990/RT	3.92	2.24
	652/605	652/605	11.58*** a	5.12

*** indicates a statistically significant difference between treatments of the same ecotype with $p<0.05$.

"a" indicates that multiple comparison testing found a significant difference between the control and high zinc treatments.

Table A5.15. Vegetation index results for each monoculture treatment over time. Kruskal-Wallis statistical test results.

Author	Formulae	JC	JL	JH	MC	ML	MH
(Blackburn, 1998)	800/675	9.5*	12.5*	12.5*	9.38*	9.38*	9.38*
	800/650	9.62*	12.5*	12.5*	9.38*	9.38*	9.42*
	800/680	9.62*	12.5*	11.58*	9.42*	9.78*	9.42*
	800/635	9.62*	12.5*	11.18*	9.35*	9.50*	10.22*
	800/500	7.44*	12.5*	11.58*	8.24*	7.94*	7.34*
	$\frac{800-680}{800+680}$	9.38*	10.22*	10.82*	9.14*	12.02*	8.88*
	$\frac{800-635}{800+635}$	9.42*	10.22*	11.58*	9.62*	12.5*	8.88*
(Carter and Miller, 1994)	695/760	12.5*	12.5*	12.02*	9.78*	9.42*	9.42*
(Malthus *et al.*, 1995)	425/470	8.34*	10.14*	10.58*	9.42*	1.49	1.25
	446/477	6.08*	7.98*	10.14*	7.22*	0.65	0.59
	541/836	9.62*	12.5*	11.18*	12.02*	6.35*	6.35*
	818/538	9.36*	12.5*	12.02*	12.02*	4.97	4.25
	818/713	9.62*	12.5*	11.58*	10.82*	4.97	4.25
(Penuelas *et al.*, 1994)	970/900	3.26	10.5*	9.26*	2.22	0.78	3.78
	$\frac{550-530}{550+530}$	9.78*	7.28*	7.02*	6.72*	6.72*	11.58*
	$\frac{680-430}{680+430}$	8.66*	9.42*	9.98*	7.02*	10.26*	7.22*
(Carter and Miller, 1994)	694/420	9.14*	12.02*	11.52*	9.5*	8.66*	9.42*
	600/760	9.62*	12.5*	12.5*	9.98*	8.82*	9.78*
	694/760	9.5*	12.5*	12.02*	9.5*	9.38*	9.38*
	VIS/760	12.02*	12.5*	12.5*	12.5*	10.64*	11.18*
(Dawson *et al.*, 1999)	NDVI	9.62*	10.82*	12.5*	11.18*	9.14*	9.38*
	SAVI	9.62*	10.28*	12.5*	10.26*	8.34*	9.38*
This Study	GP/RT	9.62*	10.5*	11.58*	7.28*	9.78*	8.78*
	GP/990	3.78	12.5*	4.25	11.18*	4.02	8.96*
	552/605	3.78	9.38*	10.5*	0.42	7.76*	2.94
	990/RT	9.42*	12.5*	12.5*	9.38*	8.66*	9.38*

* indicates a statistically significant difference between index results on different dates for that treatment with $p<0.05$.

Table A5.17. Vegetation Indices used to find a response to treatment 22/10. Kruskal-Wallis statistical test results.

Author	Name of Index	Formulae	Result
(Blackburn, 1998)	PSSRa	800/675	5.65
	PSSRb	800/650	7.42*** b
	reformed PSSRa	800/680	4.76
	reformed PSSRb	800/635	5.11
	PSSRc	800/500	1.01
	PSNDa	$\frac{800-680}{800+680}$	5.65
	PSNDb	$\frac{800-635}{800+635}$	7.42*** ab
(Carter and Miller, 1994)		695/760	7.73*** b
(Malthus *et al.*, 1995)		425/470	0.96
		446/477	2.8
		541/836	0.8
		818/538	0.73
		818/713	4.15
(Penuelas *et al.*, 1994)	WBI	970/900	5.11
	PRI	$\frac{550-530}{550+530}$	5.65
	NPCI	$\frac{680-430}{680+430}$	3.50
(Carter and Miller, 1994)		694/420	5.11
		600/760	2.8
		694/760	5.11
		VIS/760	7.53*** b
(Dawson *et al.*, 1999)		NDVI	7.42*** a
		SAVI	7.42*** a
This Study	GP/RT	GP/RT	8.00*** a
	GP/990	GP/990	2.80
	652/605	652/605	8.34*** a
	990/RT	990/RT	5.11

*** indicates a statistically significant difference between treatments with $p<0.05$.
"a" indicates that multiple comparison testing found a significant difference between the control and high zinc treatments.
"b" indicates that multiple comparison testing found a significant difference between the control and low zinc treatments.

Table A5.19. Vegetation Indices used to find a response to treatment 16/11. Kruskal-Wallis statistical test results.

Author	Name of Index	Formulae	Result
(Blackburn, 1998)	PSSRa	800/675	1.65
	PSSRb	800/650	2.34
	reformed PSSRa	800/680	1.65
	reformed PSSRb	800/635	2.42
	PSSRc	800/500	2.46
	PSNDa	$\frac{800-680}{800+680}$	1.65
	PSNDb	$\frac{800-635}{800+635}$	2.42
(Carter and Miller, 1994)		695/760	2.00
(Malthus *et al.*, 1995)		425/470	1.84
		446/477	5.65
		541/836	2.88
		818/538	1.88
		818/713	1.07
(Penuelas *et al.*, 1994)	WBI	970/900	5.11
	PRI	$\frac{550-530}{550+530}$	1.88
	NPCI	$\frac{680-430}{680+430}$	0.61
(Carter and Miller, 1994)		694/420	0.61
		600/760	4.76
		694/760	2.00
		VIS/760	4.50
(Dawson *et al.*, 1999)		NDVI	2.34
		SAVI	2.34
This Study	GP/RT	GP/RT	1.50
	652/605	652/605	1.84
	GP/990	GP/990	3.96
	990/RT	990/RT	1.50

*** indicates a statistically significant difference between treatments with $p<0.05$.

Table A5.21. Response of Vegetation Indices for the same mixture treatments over time, Mann-Whitney U test.

Author	Formulae	Control	Low Zinc	High Zinc
(Blackburn, 1998)	800/675	16*	16*	16*
	800/650	16*	16*	16*
	800/680	16*	16*	16*
	800/635	16*	16*	16*
	800/500	16*	16*	15
	$\frac{800-680}{800+680}$	16*	16*	16*
	$\frac{800-635}{800+635}$	16*	16*	16*
(Carter and Miller, 1994)	695/760	16*	16*	15
(Malthus *et al.*, 1995)	425/470	16*	16*	15
	446/477	16*	16*	13
	541/836	16*	16*	16*
	818/538	16*	16*	16*
	818/713	16*	16*	16*
(Penuelas *et al.*, 1994)	970/900	9	16*	9
	$\frac{550-530}{550+530}$	16*	16*	16*
	$\frac{680-430}{680+430}$	16*	16*	16*
(Carter and Miller, 1994)	694/420	16*	16*	16*
	600/760	16*	16*	16*
	694/760	16*	16*	15
	VIS/760	16*	16*	16*
(Dawson *et al.*, 1999)	NDVI	16*	16*	16*
	SAVI	16*	16*	16*
This Study	GP/RT	13	16*	12
	652/605	13	15	11
	GP/990	16*	16*	16*
	990/RT	16*	16*	16*

* indicates a statistically significant difference between treatments at $p<0.05$.